PHOSPHORUS CYCLING IN A PERIPHYTON-DOMINATED FRESHWATER WETLAND

By

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I dedicate this dissertation to the Mendicino and Scinto families.

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PHOSPHORUS CYCLING IN A PERIPHYTON-DOMINATED FRESHWATER WETLAND

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Periphyton, the community of microorganisms growing on submerged substrates, is a conspicuous feature of shallow, interior Everglades slough habitats. This research was conducted to identify major pathways and storages involved in P cycling. Specifically, this work centered on mechanisms functioning in unimpacted, periphyton dominated areas of the northern Everglades (Water Conservation Area 2A). The field site was dominated by calcareous blue-green algae (cyanobacteria).

Total P content was in the order of benthic (BP) > epiphytic (EP) = floating (FP) periphyton, and was in the range of 130 - 390 mg kg^{-1}. Calcium carbonate content accounted for 20 - 50 % of periphyton dry weight. Total P in BP was approximately equal to that in the surficial soil. Inorganic P (P_i), associated with Ca, was highest in the surface 0-2 cm of soil and was directly related to of increased $CaCO_3$ deposition due to calcification by BP. The presence of BP on the soil surface was shown to maintain higher soil porewater concentrations of dissolved reactive P (DRP) and Ca^{2+} than when the BP was removed.

Phosphorus uptake rates, measured in the laboratory, were 0.04 - 0.62 µmol P g^{-1} min^{-1} (dry weight periphyton) for EP and 0.02 - 0.2 µmol P g^{-1} min^{-1} for BP. Uptake parameters for EP were; V_{max} = 0.85 µmol P g^{-1} min^{-1}, K_m = 9.9 µ*M*, and for BP were; V_{max} = 0.10 µmol P g^{-1} min^{-1}, K_m = 2.5 µ*M*. Inorganic (P_i) and organic P (P_o) uptake rates by periphyton was higher under field conditions than under laboratory cultures. Both biotic and abiotic processes were shown to regulate P uptake by periphyton, with $CaCO_3$ as a barrier between living periphyton and adjacent water column. Abiotic uptake accounted for 10-30% of ^{32}P activity in one hour and 3-8% after 12 hours, suggesting that P initially associated with $CaCO_3$ surfaces is biotically incorporated with time.

In situ P uptake was greater in cores with intact BP layers (+BP) than in cores without BP (-BP). Under greenhouse conditions -BP uptake of P was initially as rapid as +BP cores. With continued P loading -BP cores lost the ability to effectively remove water column P. This suggests adsorption to soil mineral surfaces, or uptake by soil microbes can rapidly assimilate P but have limited capacity for P removal. Partitioning ^{32}P in intact cores (+BP) resulted in 14% abiotic uptake vs. 86 % biotically incorporated.

Laboratory studies were conducted to determine conditions necessary for $CaCO_3$ precipitation and subsequent P coprecipitation. Phosphorus was removed from solution when pH > 8.6 for prolonged periods. Phosphorus reduction was not observed when solution pH varied between 7.0 - 8.8 on 12 h cycles. Mineral equilibria modeling, using SOILCHEM, generally predicted hydroxyapatite as the stable mineral P form in field and reactor solutions. X-ray diffraction analysis of dried BP, and peat soil from 0-2 cm and 2-5 cm depths showed the presence of calcite but not of mineral Ca-P.

Periphyton activity controls short-term P retention via biotic uptake and creates conditions, by influencing Ca^{2+} activity, that increases long-term, stable, abiotic P retention. Loss of calcareous periphyton communities would decrease the Everglades system capacity to maintain low P concentrations.

CHAPTER 1
INTRODUCTION

Phosphorus (P), often exists in low concentrations in the hydrosphere and therefore is the component that commonly limits algal growth (Wetzel, 1983; Boström et al., 1982). Depending on the geochemical nature of the region, most uncontaminated surface waters contain between 10 to 50 μg P L^{-1} (Wetzel, 1983). Therefore, additions of P above that which a system has evolved to assimilate can have profound effects on its structure and function. Phosphorus additions to shallow waterbodies, such as wetlands and lakes, often lead to increased primary productivity due to increased algae growth. For example, increased P loads to oligotrophic wetlands can lead to changes in the community structure as was shown by Swift and Nicholas (1987) for the periphyton community in the northern Everglades. In waters that received relatively high concentrations of inorganic P and N, they observed algal communities dominated by the blue-green algae (cyanobacteria) *Microcoleus lyngbyaceus* or the green alga *Oedogonium* spp. The periphyton communities dominated by the calcareous blue-greens *Schizothrix calcicola* (Ag.) Gom. and *Scytonema hofmannii* Ag. represented low nutrient, hard water conditions (Swift and Nicholas, 1987).

Wetlands have traditionally been thought of as low-value wastelands since in their natural state they cannot be used for most agricultural activities or urban development (Lee et al., 1975). Historically, these areas have been "improved" by such "reclamation projects" as drainage for agriculture or filling for urban development (Tiner, 1984). There is little agreement as to the actual wetland acreage present at the time of this country's settlement. One estimate claims there were approximately 87 million hectares in the contiguous United States, of which an estimated 46% remains (Tiner, 1984).

The development of a global widespread environmental awareness in the last 20 years has led to an increased appreciation of the many benefits wetlands provide. The remaining wetlands of the U.S. have become a rallying point for restoration, conservation, and preservation (Kadlec and Knight, 1996). One of the most important, albeit least understood, functions of wetlands is their ability to purify water (Hammer and Bastian, 1989). This ability is derived in part from wetlands being transitional areas, found at the interface between terrestrial and aquatic systems (van der Valk et al., 1978; Tiner, 1984). Wetlands, being complex hydrologic, chemical, and biochemical systems, can store and/or transform environmentally damaging water-borne elements (Lee et al., 1975).

Phosphorus utilization in a wetland involves a complex biogeochemical cycle of interacting pathways. Since many wetlands have evolved under a limited P supply and because there is no atmospheric (gaseous) sink for P, its removal is difficult in wetland systems. On an area basis, wetlands are generally not efficient in P removal (Kadlec and Knight, 1996). An examination of the P cycle in a wetland system aids in understanding the complex interactions of pathways involved.

The cycling of phosphorus within a shallow waterbody involves numerous interactions between the P compartments of the water, biota, and soil (Fig. 1-1). Processes controlling these interactions can be biological, as in algal uptake and release, or physicochemical, such as adsorption/precipitation (Kadlec, 1987).

Phosphorus is present as inorganic (P_i) and organic (P_o) forms in wetlands. These P forms occur as both soluble and particulate forms. The relative P storage of the water is small compared to that of the biota, and much smaller relative to the soil (Boström et al., 1982; Faulkner and Richardson, 1989). Dissolved inorganic P (DIP) is the P form that is most biologically active and has the greatest impact on surface water quality (Boström et

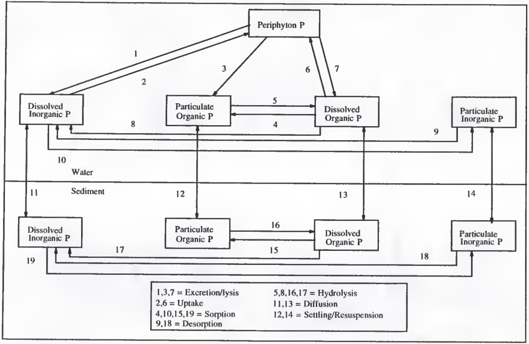

Fig. 1-1. Phosphorus cycling in a periphyton dominated freshwater wetland.

al., 1982; Syers et al., 1973). Therefore, transformations between DIP in the water and the other P components of the biota, water, and soils, and the mechanisms controlling these transformations are of ecological interest.

Dissolved inorganic P is immobilized to particulate organic P (POP) through biological uptake. Organic P, both dissolved (DOP) and POP, can account for a substantial portion of the total P in wetlands, especially those with peat soils (Richardson and Marshall, 1986). Phosphorus is bound in organic compounds as an ester (C-O-P) (Golterman, 1973; Boström et al., 1982), a form not used for autotrophic synthesis until it undergoes hydrolysis. Hydrolysis is catalyzed by phosphotase enzymes produced by bacteria and algae. Dissolved inorganic P can be retained as particulate inorganic P (PIP) by a number of abiotic processes occurring in the water and soil including, adsorption and precipitation. Dissolved inorganic P is released from PIP through desorption and dissolution. These processes are dependent upon several environmental factors, the most influential of which are pH, temperature, P content, concentration of other ions, redox potential, and the type and amount of sorbing materials (Froelich, 1988; Jacobsen, 1978; Shukla et al., 1971; Ballard and Fiskell, 1974; White and Taylor, 1977; Khalid et al., 1977; Sharpley et al., 1981). Soluble P forms move between the water and soil compartments by diffusion according to the concentration gradient. Particulate forms settle from the water and become part of the soil matrix.

Periphyton

The term periphyton is originally of Russian origin and initially referred to organisms growing on submerged, man-made objects. Eventually, it acquired a broader meaning to include the attached microorganisms, both floral and faunal, growing on natural and artificial substrates (Collins and Weber, 1978; Wetzel 1975; Browder et al., 1994). Floating mats of algae are common in many shallow waterbodies and can also be considered "periphytic", although various authors use numerous terms to differentiate between true benthic periphyton (BP) and floating algal mats. Floating periphyton (FP) has been shown to form from benthic algae that has risen in the water column because of the entrappment of photosynthetically produced oxygen bubbles (Hillebrand, 1983; Gleason and Spackman, 1974). There is considerable confusion in the nomenclature relating to periphyton (for a review see Vymazal, 1995). Periphyton can be loosely separated into types based on position in the water column although, as mentioned earlier, transformation of types can occur. The three types of periphyton considered in this research are: benthic periphyton, epiphytic periphyton (EP), and floating periphyton. Benthic periphyton grows on the soil surface. Epiphytic periphyton grows on the submerged stems of macrophytes, such as sawgrass (*Cladium jamaicense* Crantz.). Floating periphyton is that material most associated with the surface of the floodwater often in association with *Utricularia* spp.

My research is concerned with the function of periphyton or algal mats in shallow water systems and is not specifically concerned with the taxonomic classification of the multitude of autotrophic and heterotrophic organisms that compose the community. Since the basic functions of nutrient uptake, photosynthesis, and respiration are common to all, the term periphyton will be used here in a general sense.

Periphyton can be extremely important in some shallow waterbodies. The periphytic algae may contribute up to 80% of the primary productivity and therefore influence many of the biological and physicochemical aspects of these environments (Hansson, 1992; Carlton and Wetzel, 1988). Periphyton has a significant influence on P cycling. Periphyton can utilize both DIP and DOP (Bentzen et al., 1992) from floodwater and the soil porewater depending upon the vertical position of the mat in the water column (Hansson, 1989). Periphytic photosynthesis has been shown to produce marked changes in the pH and oxygen content of surficial sediments (Carlton and Wetzel, 1988), two of the more important regulators of P adsorption/desorption from sediments

(Boström et al., 1982). Periphyton is known to mediate the precipitation of $CaCO_3$ (Gleason, 1972), which can interact with adsorbed P to form more crystalline forms such as hydroxyapatite (Golterman, 1988).

Submerged and floating mats of periphytic algae are often conspicuous features of the littoral zones of lakes, slow moving rivers and streams and other shallow waterbodies (Wetzel, 1983; Swift and Nicholas, 1987). However, these mats have remained little studied when compared to pelagic phytoplankton. This is partly due to the complex nature of the periphyton communities and because of the more variable environmental physicochemical and biotic parameters of shallow waterbodies than those of the open water (Wetzel, 1983).

Need for Research

Agricultural runoff, alterations in the natural hydrology, and controlled release of water from Lake Okeechobee are causing increased levels of nutrients, most notably P, to enter into the Everglades ecosystem (Koch and Reddy, 1992; Reddy et al., 1993). This is leading to increased eutrophication of the historically oligotrophic Everglades (Swift and Nicholas, 1987).

Periphyton dominated "polishing" areas may be used as the final stage in a wetland-utilizing process for the treatment of enriched water to achieve P concentrations below those normally found in macrophyte wetlands. Since periphyton can affect the P cycle in numerous ways, it becomes important to obtain a basic understanding of the mechanisms, processes, and extent of these influences.

Site Description

The original Everglades covered an area of more than 10 000 km^2 forming a contiguous wetland system some 65 km wide and 160 km long, with water flowing from Lake Okeechobee south to the mangrove estuaries of Florida Bay (Swift, 1981). Considerable changes have occurred in the Everglades system over the last century. The original Everglades has been fragmented into a series of hydrologic units composed of the three Water Conservation Areas (WCA) and Everglades National Park. The WCAs encompass almost 3500 km^2, representing a little more than a third of the original Everglades (Swift and Nicholas, 1987). Today, southward waterflow through the series of WCAs is highly controlled by 2400 km of canals, 18 major pump stations, and hundreds of water control structures (Reddy et al., 1993).

Water Conservation Area 2A (WCA-2A) is the smallest of the three WCAs (547 km^2). It lies between WCA-1 and WCA-3A, and is heavily impacted by large quantities of nutrient enriched water derived from the Everglades Agricultural Areas (EAA) (Swift and Nicholas, 1987). The surface water quality (SWIM, 1992), nutrient gradient (Koch and Reddy, 1992), and soil nutrient characteristics (DeBusk et al., 1994) of this area have been recently studied. Water Conservation Area 2A has been shown to contain abundant calcareous blue-green algal periphyton (Gleason and Spackman, 1974).

Previous research pertaining to the floating mats of periphytic algae in the WCAs (Swift, 1981; Swift and Nicholas, 1987) focused on how environmental variables such as light, hydroperiod, and water quality factors affect the growth and species composition of the mats. The effects of periphyton on the physicochemical properties of the water column and soils, especially in relation to the P cycle have had little study.

Water Conservation Area 2A (Fig. 1-2) receives nutrient enriched water via the Hillsboro Canal (S-10C structure) causing a north to south P gradient (Koch and Reddy, 1992). In a recent study Rutchey and Vilcheck (1994) characterized about 12% of

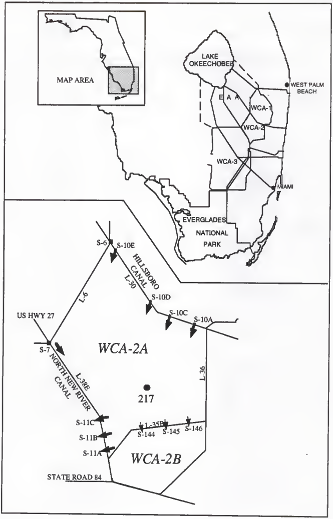

Fig. 1-2. Map of Water Conservation Area 2A (WCA) showing field site 217.

WCA-2A as being periphyton dominated. However, field observations have shown dense periphyton growth on the surface of peat and the stems of macrophytes in areas these authors characterize as being dominated by sawgrass. The site used for this research was in an area Rutchey and Vilcheck (1994) characterize as being sawgrass dominated. The effects of the P gradient are evident in the periphyton species composition, growth rate, and tissue nutrient concentrations. The dominant periphyton in the enriched water near S-10C are pollutant tolerant species such as *Microcoleus lyngbyaceous* and *Oedogonium spp.* The interior unimpacted sites are dominated by calcareous blue-green cyanobacteria such as *Schizothrix calcicola* (Ag.) Gom. and *Scytonema hofmannii* Ag., as well as hardwater diatoms. The growth rates of dominant algal species were shown to be as much as 10 times higher at enriched sites (25 mg chl a m^{-2} wk^{-1}) than at the interior sites (0.1 - 2.3 mg chl a m^{-2} wk^{-1}). The average periphyton N to P ratios at enriched sites was shown to be approximately 9:1 while at the interior sites this ratio was 107:1. The low surface water P content, periphyton tissue content, and low algal growth rates suggest the interior of WCA-2A to be P limited (Swift and Nicholas, 1987).

Objectives

The overall objective of this research was to determine the influence of periphyton on P retention in a freshwater wetland. It is hypothesized that periphyton aids in maintaining low P concentration in the water through biotic and abiotic processes. Specific objectives include:

- Determine the quantity and form of P stored in three types of periphyton, water, and soil. It is hypothesized that the quantity of P stored in periphyton depends on periphytic type, and that periphytic $CaCO_3$ production will increase the inorganic P retention capacity of the peat soil.

- Determine the P uptake kinetics of periphyton and partition uptake into biotic and abiotic processes. Phosphorus uptake should be rapid in the P limited interior of WCA-2A. Since periphyton contains both autotrophic and heterotrophic organisms both P_i and P_o should be utilized. It is also hypothesized that the $CaCO_3$ matrix of the periphyton will adsorb P_i thus providing abiotic uptake.
- Determine the influence of BP on P flux between soil porewater and the overlying floodwater. It is hypothesized that the activity of BP reduces P flux from soil porewater at ambient concentrations. Under enriched conditions, demand for P by BP should result in faster uptake as compared to periphyton-less soil. Phosphorus uptake by BP, being a function of autotrophic and heterotrophic organisms, should occur under light and dark conditions.
- Determine the physicochemical conditions in the water column which lead to P retention in stable mineral forms. It is hypothesized that high pH conditions causes the precipitation of $CaCO_3$ and coprecipitation of P and that high CO_2 causes dissolution of the solids.
- Assess in situ ion activities and physicochemical conditions to predict stable mineral forms.

Data were generated from field, laboratory, and greenhouse experiments. Chapter 2 provides a characterization of the system. Phosphorus uptake kinetics using laboratory and in situ batch incubations are presented in Chapter 3. Phosphorus flux between soil porewater and floodwater was studied in intact cores in the field and greenhouse, and these data are discussed in Chapter 4. Chapter 5 presents a laboratory reactor study using WCA-2A-217 water incubated at varying pH to determine precipitation processes and presents results of geochemical modeling for laboratory and in situ conditions. Finally, Chapter 6 is a summary synthesizing the significance of the results to P retention in the Everglades.

CHAPTER 2
PHYSICOCHEMICAL PROPERTIES OF PERIPHYTON, SOIL, WATER, AND VEGETATION

Introduction

Phosphorus in wetlands exists in a state of dynamic equilibrium, being distributed between numerous compartments including periphyton, vegetation, water, and soil. The system is complex due to the number of storage compartments, each having different uptake and release mechanisms (Howard-Williams, 1985; Kadlec and Knight, 1996; Richardson and Marshall, 1986). The various compartments contain inorganic P (P_i) and organic P (P_0) in dissolved and particulate forms. The availability of P for biotic utilization depends on the compartment and form of P contained. The dissolved inorganic P (DIP) is the form that is most biologically active and therefore is in greatest demand. The biological productivity of many aquatic systems is dependent upon how quickly this form of P can be cycled through the system (Wetzel, 1983). Other forms of P, especially recalcitrant P_0, tend to accumulate in soils and can account for a substantial fraction of the total P (Koch and Reddy, 1992; Faulkner and Richardson, 1989).

The proportion of P in various compartments varies depending on the stability and lability of stored P. The relative P storage in the water compartment is small compared to that of biota and soil (Boström et al., 1982; Faulkner and Richardson, 1989; Richardson and Davis, 1987; Verhoeven, 1986). Phosphorus in the water compartment is affected by numerous pathways and generally has a short residence time. Periphyton and microorganisms are responsible for the initial uptake of DIP from the water column, while vegetation provides short-term storage of P in the form of biomass (Correll et al., 1975; Howard-Williams and Allanson, 1981; Richardson and Marshall 1986). Periphyton removes DIP from the water column and converts it into particulate organic P (POP) in the form of biomass. Periphyton is short-lived and can return the DIP removed for cell growth back to the water column in the form of POP. In oligotrophic or P-limited systems, P released from dead cells is rapidly recycled back into the periphyton such that the P stored in the periphyton layers is maintained at a semi-constant content (Wetzel, 1993). Emergent macrophytes and other aquatic vegetation tend to store P on a seasonal basis, releasing large quantities of stored nutrients at senescence. The soil represents the largest storage compartment and generally retains P on longer time scales than the other storages. Dissolved P is labile and readily moves between the soil interstitial water and the water column by diffusion according to the concentration gradient (Reddy et al., 1996)

Phosphorus, as compared to other biotically essential elements, often exists in low concentrations in the hydrosphere and therefore limits primary productivity (Wetzel, 1983; Boström et al., 1982). Depending on the geochemical nature of the region most natural surface waters contain between 10 and 50 μg P L^{-1} (Wetzel, 1983). The size of the water storage is dependent upon the water depth and P concentration. Interactions of water-borne P with the uptake mechanisms of periphyton and soil depend largely upon residence time of water over a given surface area of wetland. Emergent macrophytes influence water storage by slowing flow thus increasing the residence time and allowing greater removal of floodwater P (Howard-Williams, 1985).

Periphyton can be defined as the attached microorganisms, both floral and faunal, growing on submerged natural or artificial substrates (Collins and Weber, 1978; Wetzel, 1983). Periphyton can be loosely separated into types based on position in the water column although transformation of types occur. The water column position determines the sources of nutrients available to the periphyton. Benthic periphyton (BP) is composed of the microcommunities of autotrophic and heterotrophic microorganisms associated with the flooded soil surface. Benthic periphyton often coats the soil surface with a loose blanket of material or with a cohesive mat. Benthic periphyton has been shown to obtain P from the overlying water column and from the soil interstitial water (Hansson, 1989). This type of periphyton is also associated with the detrital layer of the wetland. Particulate matter settling from the water column passes into the BP layer. Epiphytic periphyton (EP) grows on the submerged surfaces of emergent macrophytes such as sawgrass (*Cladium jamaicense* Crantz.). The EP receives most of its P supply from the water column. Small amounts of P from living macrophyte exudates can augment floodwater P, but this amounts to a small fraction of periphytic P (Riber et al, 1983; Carignan and Kalff, 1982). Upon senescence a substantial amount of macrophyte P can be released. The proximity of EP aids in securing the P before it is diluted into the surrounding water column (Wetzel, 1983). Floating periphyton (FP) receives P solely from the water column.

The soil compartment contains the largest storage of P in wetlands. Almost all of the P stored in the soil is held in mineral inorganic forms or in resistant organic forms and is not readily available for biotic use. For example, Verhoeven (1986) found only 0.2 - 3.0% of the TP in minerotrophic peat mires was in an available form. Forms of P present in soils and sediments have been characterized by their differential solubilities in chemical extractants. Procedures for chemical fractionation of soil P are developed with the intention of sequentially removing discrete pools of P. Generally, the biologically available P is removed first, followed by labile P_i and P_o, and then more stable forms of inorganic and organic P. The terminology used to describe the various extracted P pools is mostly operational (Psenner and Pucsko, 1988). Numerous extractants have been used to remove P from mineral soils (Chang and Jackson, 1957; Hedley et al., 1982; Hedley and Stewart, 1982), organic soils (Walbridge and Vitousek, 1987), and freshwater (Olila et al., 1995; Williams et al., 1971; Frink, 1969; Syers et al., 1973) and marine (Hieltjes and Lijklema, 1980; van Eck, 1982) soils/sediments. Several extraction procedures have been recently applied to peat soils of the Florida Everglades (Koch and Reddy, 1992; DeBusk et al., 1994; Qualls and Richardson, 1995). The extraction scheme presented by Hedley et al. (1982) separates the P fractions into i) readily biologically available, ii) labile P_i and P_0 sorbed on the soil surface, iii) labile P_0, vi) chemisorbed Fe and Al-P, v) internal soil particulate P_i, vi) apatite-type minerals, and vii) resistant P forms. Modifications of this procedure have been used on Everglades peat soils (Qualls and Richardson, 1995).

Long-term storage of P in a wetland is limited. Leaf litter, decaying vegetation and periphyton generally decompose to stable residuals in time spans of less than 24 months, depending upon numerous factors including microbial activity and temperature. The residual material is a small portion of the original biomass. Therefore standing biomass cannot be counted as a long-term, sustainable P removal mechanism (Richardson, 1985). Soil retention of P is limited by the sorption capacity of the soil unless new sites are formed. The soil compartment saturates unless there is an input of new soil materials (Richardson, 1985). The two possible mechanisms for sustainable P retention are the accretion of biomass residuals and the formation or accumulation of mineral forms (Richardson, 1985). Peat soils can accumulate P in long-term storage by the accretion of organic matter (Reddy et al., 1993; Richardson and Craft, 1993). In systems with high calcium activity, photosynthesis induced pH changes can cause the precipitation of $CaCO_3$ and the coprecipitation of P (Otsuki and Wetzel, 1972) which is subsequently deposited on the soil surface. The Ca-P complex is eventually incorporated into the soil as shown by the linear relationship between long-term Ca and P accumulation in the Everglades system (Reddy et al., 1993).

Peat soils are the largest reservoir of nutrients, including P, in the Everglades system. The accretion of peat has been shown to regulate the long-term P storage of the Everglades (Reddy et al., 1993; Craft and Richardson, 1993). The accretion of peat soils is dependent upon a number of environmental factors including hydrology and nutrient regimes. Peat accretion rates and corresponding nutrient accumulation rates have been estimated in wetland systems using ^{137}Cs as a marker (DeLaune et al., 1978). This method was recently used in several studies of peat accretion in the Water Conservation Areas (WCAs) of the northern Everglades (Craft and Richardson, 1993; Reddy et al., 1993).

The objective of this study was to describe the major P storages in an oligotrophic, periphyton dominated, wetland with peat soils. An area sparsely vegetated with sawgrass of the northern Everglades will serve as an example. Knowing the sizes and forms of stored P allows inferences to be made as to the potential mobility of stored P, estimates of P accumulation rates, and identification of the mechanisms controlling long-term P retention.

Materials and Methods

Site Description

Soil, water, periphyton, and vegetation characterized in this study were collected from an interior site, designated 217, in Water Conservation Area 2A (WCA-2A-217) (26° 17.17' N, 80° 24.73' W ± 30 m) of the Florida Everglades. The hydrology of WCA-2A is intensively managed to provide water supply during the dry season and to provide water storage during the rainy season. This area receives nutrient laden water across its northern border via the Hillsboro Canal (Fig. 2-1). Steady external nutrient loading has resulted in a P concentration gradient in the soil and water column with higher P concentrations near inflow control structures and background P contents at interior sites (SWIM, 1992; Koch and Reddy, 1992). The interior site of WCA-2A-217 is characterized by peat soils, patchy sawgrass stands, and the presence of calcareous

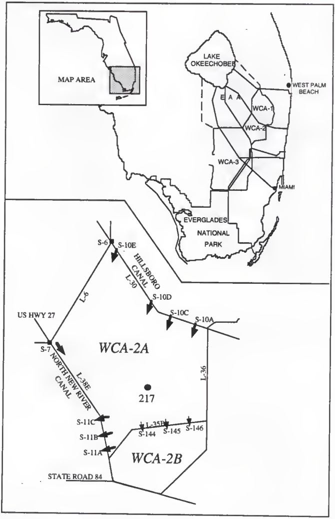

Fig. 2-1. Map of Water Conservation Area 2A (WCA) showing field site 217.

periphyton; covering most of the peat, coating the submerged portions of older stems of sawgrass, and occasionally forming floating mats on the water surface (Swift and Nicholas, 1987).

Water Sampling and Analysis

Bulk water samples were collected from the surface floodwater on April 10, 1995. The water depth averaged 0.6 m on the sampling date. Field-filtered and unfiltered samples were collected near the water surface in the proximity of mats of FP. Water samples were stored in polyethylene bottles and submerged in ice water until returned to the laboratory. Samples were stored at 4° C until analyzed. Unfiltered water samples were analyzed for pH, electrical conductivity (EC) (Method 2510, APHA, 1992), alkalinity (Method 2320, APHA, 1992), and TP after persulfate oxidation (Method 4500-P-B, APHA, 1992). Field-filtered (0.2 µm membrane filters; Gelman Supor, Gelman, Inc.) water samples were split before analysis. One subsample was analyzed colorimetrically for dissolved reactive P (DRP) (Ascorbic Acid Method 4500-P-E, APHA, 1992) on an AutoAnalyzer II (Technicon Corp.). The other subsample was digested and analyzed colorimetrically for total dissolved P (TDP). Total C (TC), total organic C (TOC), and total inorganic C (TIC) were analyzed on unfiltered samples on a DC-190 High-Temperature TOC Analyzer (Rosemount Analytical Inc. Dohrmann Division, Santa Clara, CA). Filtered water samples were analyzed for the cations: Ca, Mg, K, Zn, Na, Cl, Si, Cu, Mn, Al, and Fe using Inductively Coupled Argon Plasma Spectrometry (ICP) (Method 3120B, APHA, 1992). Sulfate-S and NO_3-N were analyzed by ion chromatography on filtered samples using a Dionex 4500i (Dionex Corp. Sunnyvale, CA.)(Method 4110, APHA, 1992).

Periphyton and Macrophyte Sampling and Analysis

Benthic periphyton was sampled on August 12, 1993 and on April 10 and 21, 1995. Benthic periphyton was collected by removing the material trapped in a 14.5 cm I.D. acrylic core. Three replicate samples were collected during the 1993 sampling while four replicates were collected in 1995. In April 1995, BP was collected in an area sparsely populated with sawgrass. This BP was termed $BP_{sawgrass}$. Additionally, BP samples were also collected from an open water area (slough) in the same vicinity. These samples were designated BP_{slough}. Epiphytic periphyton was collected by clipping sawgrass at the floodwater/benthic periphyton interface and at the air/water interface. The macrophyte and EP were returned to the laboratory where they were separated by gently scraping the EP with a scalpel. Four clusters of heavily colonized plants were sampled to provide replication. The separated sawgrass stems were then sectioned into below water living and dead material, and above water live material. Floating periphyton was collected with a swimming pool net from four floating mats.

Periphyton and sawgrass materials were oven-dried at 70° C to constant weight for the determination of water content. Some of this material was then combusted in a muffle furnace at 450° C for 12 h to determine ash content. This modification of standard practice (550° C for 4 h) was done because of the dolomitic nature of the $CaCO_3$ suspected in the dried material which begins to degrade at temperatures in the 550° C range. The objective was to remove all organic matter while leaving mineral carbon forms intact. Recoveries of standard materials, which included $CaCO_3$, dolomite, and estuarine sediment, showed the modified ashing method to yield results within 10% for the two methods. Oven-dried subsamples were ground and digested by perchloric acid (Method 4500-P-B, APHA, 1992) for colorimetric analysis of total P. This solution was analyzed for cations (Ca, Mg, Fe, and Al) by Flame Atomic Absorption (FAA) (Method 3111, APHA, 1992). Total C (TC) and total N (TN) were determined on 1 to 5 mg finely ground (#100 mesh) oven-dried subsamples and analyzed using a Carlo-Erba NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, New Jersey) (Nelson and Sommers, 1982). Total inorganic C (TIC) was determined on ashed material (as above using the CNS analyzer). Total organic C was determined by difference. The $CaCO_3$ content was calculated assuming that all TIC came from $CaCO_3$. Multiplying TIC by 8.33 (mole percentage of C in $CaCO_3$) yields an estimate of $CaCO_3$ content.

Benthic periphyton (air dried, ground (#100 mesh)) samples were extracted with 1.0 *M* HCl in a 1:50 BP to solution ratio. After a 3 h equilibration under continuous shaking, samples were centrifuged (6000 rpm, 15 min) and the supernatant filtered through 0.45 µm membrane filters. Filtered solutions were analyzed colorimetrically for DRP and by Flame Atomic Absorption for extracted cations, as described above.

Separate subsamples of field moist BP were extracted with 0.5 *M* $NaHCO_3$ at a solid to solution ratio of 1:100. The suspensions were equilibrated for 16 h under continuous shaking, then centrifuged at 7000 rpm for 15 min. Supernatant solution was filtered through 0.45 µm membrane filter (Gelman Supor, Gelman, Inc.) and analyzed colorimetrically for DRP. A second subsample of field moist was treated with 1 ml of $CHCl_3$, vortexed, and allowed to evaporate overnight. This was followed by extraction with 0.5 *M* $NaHCO_3$, as described above. Extracted solutions were analyzed for DRP and TP The difference between $CHCl_3/NaHCO_3$-TP and $NaHCO_3$-P_i was considered labile P_o. When this extraction was used on agricultural soils, the additional P extracted after $CHCl_3$ treatment was shown to originate from lysed microbial cells (Hedley and Stewart, 1982).

Soil Sampling and Analysis

Soil samples were collected by slowly driving a 10 cm I.D., thin-walled aluminum tube, with a sharpened edge, into the peat soil. On August 12, 1993, four replicate soil cores were collected to a depth of 20 cm and were sectioned into 10 depth increments (cm): 0-1, 1-2, 2-3, 3-4, 4-5, 5-7, 7-9, 9-12, 12-15, 15-20. On April 10, 1995, four replicate cores were collected to a depth of 5 cm and sectioned into 0-2 and 2-5 cm layers.

Soil pH was determined by taking a 5 g homogenized subsample of each field moist soil sample and mixing with 5 g distilled, deionized water (DDI-H_2O) to achieve a 1:1 soil slurry. The pH was obtained using a combination glass electrode and a standard pH meter. A known mass subsample of the homogenized soil was dried to constant weight at 70° C and bulk density was determined (g dry weight soil cm^{-3}). The procedure used follows that of Blake and Hartge (1986), with the exception that soils were dried at 70° C instead of at 105° C. The alteration of standard method was done considering the organic nature of these soils. Subsamples of air dried, ground (#100 mesh), and weighed (approximately 0.2 g) soil was digested with perchloric acid and analyzed for TP as above. Dried, ground (#100 mesh) soil was extracted with 1 *M* HCl in a 1:50 soil to solution ratio and analyzed for P_i and selected cations, as previously described. Dried soil samples were used to determine the TC, TIC, TOC, and $CaCO_3$ content following the procedures above.

Fractionation of Soils and Benthic Periphyton

The soil and BP samples, collected in 1993, were fractionated to determine discrete pools of P using a modification of the extraction scheme as presented in Hedley et al. (1982). The procedure used (Fig. 2-2) modified the original scheme by omitting the initial removal of exchangeable P with anion exchange resin. The sonication and second 0.1 *M* NaOH extraction were also omitted from our scheme. This fraction is meant to represent P held at the internal surfaces of soil aggregates (Hedley et al., 1982). Residual P was not determined by digestion of sequentially extracted soil, but rather as

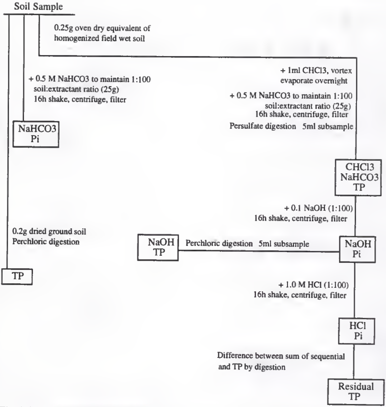
Soil Sample
0.25g oven dry equivalent of
homogenized field wet soil
+ 0.5 M NaHCO3 to maintain 1:100
soil:extractant ratio (25g)
16h shake, centrifuge, filter
NaHCO3
Pi
+ 1ml CHCl3, vortex
evaporate overnight
+ 0.5 M NaHCO3 to maintain 1:100
soil:extractant ratio (25g)
16h shake, centrifuge, filter
Persulfate digestion 5ml subsample
CHCl3
NaHCO3
TP
+ 0.1 NaOH (1:100)
16h shake, centrifuge, filter
0.2g dried ground soil
Perchloric digestion
NaOH
TP
Perchloric digestion 5ml subsample
NaOH
Pi
TP
+ 1.0 M HCl (1:100)
16h shake, centrifuge, filter
HCl
Pi
Difference between sum of sequential
and TP by digestion
Residual
TP

Fig. 2-2. Diagrammatic representation of phosphorus extraction procedure.

the difference between the sum of the sequentially extracted P and TP by perchloric acid digestion. Additionally, the 0.1 *M* NaOH extract was digested for TP.

Field moist soils were simultaneously extracted with 0.5 *M* $NaHCO_3$ and 0.5 *M* $NaHCO_3$ following $CHCl_3$ treatment. Phosphorus extracted with $NaHCO_3$ was considered labile P_i and represents plant available P (Olsen and Sommers, 1982). Phosphorus extracted with $NaHCO_3$ after $CHCl_3$ treatment is composed of the labile P from above, surface sorbed P_o, and a portion of the P released from lysed microbial cells (Hedley et al., 1982). Hedley and Stewart (1982) found that digested extracts of $CHCl_3/NaHCO_3$ minus the P recovered solely from $NaHCO_3$ extraction represented about 40% of the microbial P they added to their soil. I consider the P release by $CHCl_3/NaHCO_3$ minus the labile P_i to represent labile P_o. The residual soil from the labile P_o extraction above was treated with 0.1 *M* NaOH to obtain a soil to solution ratio of 1:100. Soil suspensions were equilibrated for 16 h under continuous shaking, then centrifuged at 7000 rpm for 15 min. Supernatant solution was filtered and analyzed for DRP as above. The NaOH-extractable P, as analyzed colorimetrically for DRP, is considered to be Fe/Al-bound P_i (Ryden et al., 1977; van Eck, 1982). Hydrolyzable P_o was obtained by digestion of a subsample of NaOH extracted P (yielding TP) minus the Fe/Al-bound P_i. This fraction is considered to be moderately resistant organic P associated with humic and fulvic acids (Olila et al., 1995). Phosphorus extracted with 1 *M* HCl represents Ca-bound P_i (Williams et al., 1971). This P is associated with apatite or apatite-like minerals, essentially as calcium phosphate. Phosphorus can also be present in amorphous and transitional forms such as monocalcium, dicalcium, and octacalcium phosphates. Unless soil conditions become acidic this fraction of P is not readily solubilized (van Eck, 1982). Residual P (as the difference between perchloric acid TP and sum of extracted forms) is the P remaining in soil at the end of the sequential extraction and represents resistant organic and mineral P forms.

Peat Accretion Rates

Four replicate soil cores for ^{137}Cs analysis were collected in the same manner as cores used in soil fractionation. These cores were sectioned by 1 cm increments to a total depth of 20 cm. Soil sections were air dried, weighed, and ground to pass a 1 mm sieve. Vertical peat accretion rates were estimated by measuring the ^{137}Cs distribution in the soil profile (DeLaune et al., 1978). The ^{137}Cs activity in each section was determined by gamma counting oven-dried soils with a germanium detector and multichannel analyzer. ^{137}Cesium entered the atmosphere as a by-product of aboveground thermonuclear testing. This ^{137}Cs was then deposited on the soil surface as fallout. The first noticeable levels of ^{137}Cs appeared in 1954: the maximum level corresponds to the soil surface of 1964 (Pennington et al., 1973). Therefore ^{137}Cs provides a 1964 time signature in the peat soil. The accumulation of peat above the 1964 ^{137}Cs peak can be considered peat deposited in the past 29 years. From this information, peat accretion and the accumulation rates of P and selected chemical components were calculated. Peat accretion rate (PAR) was calculated as

$$\text{PAR} = \text{137Cs peak depth (mm) / Number of years since 1964} \qquad [2\text{-}1]$$

where PAR = peat accretion rate (mm yr^{-1}) (converted from cm depth increments), and Number of years since 1964 = 29. Accumulation rates (AR) (mg m^{-2} yr^{-1}) for P and other components were calculated by

$$\text{AR} = [(\Sigma C\ \rho d\ 1\ \text{cm}) / n]\ 10^4 \text{ for mg m}^{-2}\text{ yr}^{-1} \text{ or } 10 \text{ for g m}^{-2}\text{ yr}^{-1} \qquad [2\text{-}2]$$

where AR = accumulation rate in mg m^{-2} yr^{-1} or g m^{-2} yr^{-1}, C = concentration (mg kg^{-1}), ρd = bulk density for each 1 cm depth increment (g cm^{-3}), n = number of years since 1964 (n = 29 yr), and 10^4 or 10 to convert to m^2 basis.

Statistical Analysis

Differences in chemical composition of periphyton types (Table 2-2) and sawgrass (Table 2-3) were analyzed statistically using a one-way analysis of variance. Where appropriate, the level of significance (p-value) is designated (p-value; *** = 0.001, ** = 0.01, * = 0.05, ns > 0.05). Sample means determined to be significantly different (α = 0.05) were evaluated by Tukey's Studentized Range Test. Values given in tables followed by similar letters are not significantly different. Correlation analysis was performed on physicochemical characteristics of periphyton and soil samples collected on August 12, 1993. The correlation coefficients (r) and level of significance of the correlation (p-value) are given for important relationships. All statistics were performed on SAS Proprietary Software Release 6.08 (SAS Institute Inc. 1989, Cary NC.)

Results and Discussion

Water

Surface water of the interior WCA-2A site was characterized by relatively high electrical conductivity, a basic pH, and high alkalinity (Table 2-1). The major anions were Cl and HCO_3^- . Major cations included Na, Ca, and Mg. These results generally agree with those of Swift and Nicholas (1987) measured at this site between 1977 and 1983, except that the pH measured in our study was almost one unit higher, and SO_4 about half their average. Swift and Nicholas (1987) suggest the highly mineralized nature is due to the influx of high ionic strength water from agricultural production in the EAA. Total inorganic C and TOC each represent about 50 % of the TC in the water column.

Swift and Nicholas (1987) describe the interior of WCA-2A as being a minerotrophic peatland. Minerotrophic peatlands develop in areas that receive mineral enriched water inputs by draining alkaline soils or that overlie mineral soils. The bedrock

Table 2-1. Chemical composition of WCA-2A-217 surface water collected on April 10, 1995, (n=5 unless noted† then n=4).

Parameter	Units	Mean ± SE
pH (mean and range)		7.93 (7.85 - 8.20)
Electrical Conductivity	mS m^{-1}	83.2 ± 0.7
Alkalinity (CaCO$_3$ to pH=4.5)	mg L^{-1}	230 ± 2
Dissolved Reactive P	μg L^{-1}	9.4 ± 0.1
Total Dissolved P	μg L^{-1}	10.7 ± 2.1†
Total P	μg L^{-1}	10.8 ± 0.7
Total C	mg L^{-1}	94.2 ± 1.5
Total Inorganic C	mg L^{-1}	45.5 ± 0.5
Total Organic C	mg L^{-1}	48.7 ± 1.9
Sulfate - S	mg L^{-1}	18.0 ± 0.2
Ca	mg L^{-1}	64.6 ± 0.5
Mg	mg L^{-1}	32.1 ± 0.1
K	mg L^{-1}	6.9 ± 0.1
Zn	mg L^{-1}	0.3 ± 0.1
Na	mg L^{-1}	106 ± 0.2
Cl	mg L^{-1}	164 ± 1
Si	mg L^{-1}	7.6 ± 0.1
Cu, Mn, Al, and Fe	mg L^{-1}	< 0.1
Nitrate - N	mg L^{-1}	< 0.2

underlying the peat soil at WCA-2A-217 is calcareous limestone of the Ft. Thompson formation (Gleason, 1972). Characteristics of minerotrophic peatlands are alkaline conditions, hard water, bicarbonate as a dominant anion, high Ca concentrations, neutral to basic pH, high EC, and low concentrations of inorganic nutrients (P) (Moore and Bellamy, 1974).

Dissolved reactive P was 10 $\mu g\ L^{-1}$ and accounted for nearly 90 % of TP. The ratio of DRP to TP is expected as the hydrolysis of organic P compounds, represented by ATP, is known to be rapid (see Chapter 3). The TP values obtained agree with those of Swift and Nicholas (1987), but they obtained DRP concentrations $< 2\ \mu g\ L^{-1}$ or < 20% of their TP was DRP. Qualls and Richardson (1995) found DRP (SRP) to comprise nearly 80% of TP at their unenriched sites in WCA-2A; however, their TP averaged 36 $\mu g\ L^{-1}$ or about 3 times the TP concentrations in our study. Concentrations of water P forms in the Qualls and Richardson (1995) study are averages of samples collected from several transects, some of which are in closer proximity to drainage canals than was the site used in this study.

Total P content in the water column is a function of P inputs: through precipitation, by the influx of P from upstream sources, and by the cycling of P between P containing compartments at the site. Although WCA-2A receives an estimated 5.4×10^4 kg P yr^{-1} (South Florida Water Management District, 1991) as a function of the pumping of nutrient laden water from the Everglades Agricultural Area (EAA), this P has been shown to be removed from the water column upstream of WCA-2A-217 and does not affect TP concentration at this site (Koch and Reddy, 1992). If TP concentrations are considered to be relatively constant (as based on similarity in TP of Swift and Nicholas, 1987 study and results of this study), then the P cycle may be described as closed to outside inputs. In a steady-state system, where nutrient inputs from outside the system are minor compared to consumption rates, nutrient regeneration from within the system becomes more important to overall system productivity (Howard-Williams, 1985).

Nutrient recycling rates are a function of the proportion of available nutrient to unavailable form. Dissolved reactive P is the most biologically available form; therefore, in a system with rapid recycling of nutrients it would be expected that mechanisms (e.g. alkaline-phosphotase production) would be developed to increase the proportion of DRP to TP. The high (90%) proportion of TP that was DRP from this analysis suggests nutrient recycling at WCA-2A-217.

Periphyton

In WCA-2A-217 BP was associated with the peat substrate under sparsely vegetated sawgrass ($BP_{sawgrass}$ and BP collected in August 1993 (BP_{93})), and in open sloughs (BP_{slough}), EP was growing on sawgrass stems, and FP was present at various positions in the water column, usually near the water surface. Benthic periphyton consisted of loose accumulations of photosynthetic, mineral, and detrital organic materials. Although stratification within the BP layers undoubtedly occurred (see pH profiles Chapter 5) the BP at this site is best described as an integrated periphyton type rather than a biofilm mat according to the descriptions given by Wetzel (1993). The growth of EP was much greater on submerged, dead sawgrass stems than on living stems. Similarly, Browder (1981) found preferential colonization on dead macrophyte stems. Floating periphyton occasionally colonized *Utricularia spp.* When this occurred, the *Utricularia spp.* was often completely covered and accounted for a very small portion of the floating material.

Several similarities exist between $BP_{sawgrass}$ collected in 1995 and the BP collected in 1993, reflecting the stable nature of the interior of the northern Everglades. Samples collected were from the same general area. The TP contents generally agreed, with $BP_{sawgrass}$ being higher but not statistically different from BP_{93} (Table 2-2). Water

Table 2-2. Characterization of benthic (BP), epiphytic (EP), and floating (FP) periphyton collected at WCA-2A-217 in August, 1993 (BP_{93}) and April, 1995. In April 1995, BP was collected from a location sparsely vegetated with sawgrass ($BP_{sawgrass}$) and from an open slough (BP_{slough}) in the same vicinity. The BP_{slough} values are the averages of one sample collected April 10, 1995 and three samples collected April 21,1995 (n = 4, unless noted † then n = 3, if noted ‡ then only April 10 BP_{slough} sample analyzed). Levels of significantly different sample means are given by p-values (***= 0.001, **= 0.01, *= 0.05, ns > 0.05). Values followed by similar letters were not significantly different.

Periphyton Type	Units	p-value	August, 1993 BP_{93}	April, 1995 $BP_{sawgrass}$	BP_{slough}	EP	FP
Parameter	(dry wt. basis)		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Total P	mg kg^{-1}	***	330 ± 5^{a}	385 ± 13^{a}	385 ± 71^{a}	157 ± 10^{b}	132 ± 10^{b}
Total Ca	g kg^{-1}	***	NA	260 ± 19^{b}	542 ± 61^{a}	412 ± 14^{a}	502 ± 17^{a}
Total Mg	g kg^{-1}	**	NA	7.17 ± 0.41^{b}	9.96 ± 0.84^{a}	7.69 ± 0.37^{b}	8.81 ± 0.08^{ab}
Total Al	g kg^{-1}	***	NA	1.19 ± 0.07^{a}	1.01 ± 0.18^{a}	0.16 ± 0.02^{b}	0.24 ± 0.03^{b}
Total Fe	g kg^{-1}	***	NA	1.72 ± 0.19^{a}	1.05 ± 0.19^{b}	0.40 ± 0.02^{c}	0.26 ± 0.02^{c}
Water Content	%	***	$93.6 \pm 0.3†^{a}$	93.5 ± 0.3^{a}	95.8 ± 0.5^{b}	97.1 ± 0.2^{b}	96.9 ± 0.2^{b}
Ash Content	%	**	33.4 ± 2.1^{b}	32.6 ± 2.1^{b}	61.1 ± 10.4^{a}	50.8 ± 2.2^{ab}	53.4 ± 0.8^{ab}
Total N	g kg^{-1}	***	NA	25.4 ± 0.6^{a}	22.1 ± 0.7^{b}	12.0 ± 0.5^{c}	10.9 ± 0.5^{c}
Total C	g kg^{-1}	***	321 ± 38^{ab}	349 ± 7^{a}	279 ± 6^{abc}	251 ± 4^{bc}	220 ± 1.8^{c}
TC:TN		***	NA	13.7 ± 0.2^{b}	12.6 ± 0.1^{b}	21.0 ± 0.5^{a}	20.3 ± 0.7^{a}
Total Inorganic C	g kg^{-1}	**	26.0 ± 2.2^{b}	23.6 ± 2.4^{b}	48.9 ± 9.5^{a}	38.6 ± 2.8^{ab}	38.8 ± 1.3^{ab}
Total Organic C	g kg^{-1}	***	295 ± 37^{ab}	325 ± 10^{a}	230 ± 12^{bc}	212 ± 7^{c}	182 ± 3^{c}
$CaCO_3$	g kg^{-1}	**	217 ± 19^{b}	197 ± 20^{b}	408 ± 78^{a}	321 ± 23^{ab}	323 ± 11^{ab}
Bulk Density	g cm^{-3}	***	$0.011 \pm <0.001^{a}$	0.037 ± 0.002^{b}	$0.029‡^{b}$	NA	NA
Labile P_i	mg kg^{-1}	***	$1.8 \pm 0.6†^{b}$	13.0 ± 1.1^{a}	NA	NA	NA
Labile P_o	mg kg^{-1}	***	$38 \pm 1†^{b}$	115 ± 7^{a}	NA	NA	NA
1 M HCl extractable P_i	mg kg^{-1}	ns	$130 \pm 2†$	172 ± 3	168 ± 22	NA	NA
1 M HCl extractable Ca	g kg^{-1}	***	113 ± 4^{a}	176 ± 8^{b}	$289‡^{c}$	NA	NA
1 M HCl extractable Mg	g kg^{-1}	ns	4 ± 0.1	4.2 ± 0.1	4.3‡	NA	NA
1 M HCl extractable Al	g kg^{-1}	ns	0.31 ± 0.09	0.25 ± 0.02	0.30‡	NA	NA
1 M HCl extractable Fe	g kg^{-1}	***	0.34 ± 0.02^{a}	0.23 ± 0.01^{b}	$0.20‡^{c}$	NA = not available	

content, ash content, TC, TOC, TIC, $CaCO_3$ content, and 1 *M* HCl extractable Mg, and Al were all virtually the same for the $BP_{sawgrass}$ and BP_{93}. The dissimilarities between $BP_{sawgrass}$ and BP_{93} appear in the bulk densities and in the concentrations of labile P_i, labile P_o, and HCl extractable P_i and Ca.

For the periphyton collected in 1995 the BP contained over twice as much TP than did EP, or FP (Table 2-2). Total P content was in the order $BP_{sawgrass} = BP_{slough} > EP = FP$. Similarly, TC and TN followed these trends, except that $BP_{sawgrass}$ had higher concentrations than all other types. Total C in BP_{slough}, EP, and FP were similar while that of $BP_{sawgrass}$ was generally higher. The increase in TC of $BP_{sawgrass}$ was the result of higher TOC concentration and not TIC. Both BP types had about twice the TN content of EP and FP, resulting in C:N of half that of EP and FP. The higher TC and TN contents reflect the increased supply of organic C and N available to BP from decaying sawgrass litter. The ash content, total Ca, and $CaCO_3$ content were greater in the BP_{slough} samples. The total Ca contents of all periphyton forms were extremely high with total Ca accounting for around 50% of the total dry mass of periphyton. If this Ca was associated only with $CaCO_3$, then the $CaCO_3$ contents would account for 65%, 136%, 103%, and 126% of the total dry weight of $BP_{sawgrass}$, BP_{slough}, EP, and FP, respectively. Ash content was > 50% for all periphyton types except $BP_{sawgrass}$. Total inorganic C was 18% of the TC in FP, 15% in EP, 18% in BP_{slough}, and 7% in $BP_{sawgrass}$. Concentrations of Fe and Al were low in all periphyton types, especially EP and FP. The Mg concentrations were similar for all periphyton types and followed the same order as total Ca ($BP_{slough} > FP > EP > BP_{sawgrass}$).

Sawgrass

The most dramatic difference between live and dead portions of sawgrass was that of TP content (Table 2-3). Live sawgrass contained 5 - 7 times more P than dead stems. There was not a corresponding decrease in TN or TC between submerged live and dead stems. The TC:TN was about 130 in both cases. This suggests that upon senescence a considerable portion of the plant P was either lost to the water column through leaching or incorporated into the EP. There was no evidence for a similar transfer of TC or TN. Davis (1990, as reported in Table 14-2 of Kadlec and Knight, 1996) reported P contents of 400 mg kg^{-1} in live material and 200 mg kg^{-1} in dead sawgrass and litter material from the oligotrophic Everglades areas he studied. The TP concentrations in the live sawgrass analyzed in this study were about 1/2 and the dead sawgrass contained about 1/7 those reported by Davis (1990). Soluble substances are rapidly leached from senescent macrophytes. Leaching was shown to cause the loss of 50% of the P content in *Typha lattifolia* L. in the first three weeks following senescence (Kulshreshtha and Gopal 1982). Nitrogen content did not behave in this manner; little change in N content was observed between live and dead plant material (Kulshreshtha and Gopal 1982). It should be noted that the water content of submerged dead sawgrass was much greater than that of living stems. The implications of this are that much larger surface areas of

dead material contributed to the analyzed concentrations of nutrients. Epiphytic periphyton growing on the surface of dead stems would therefore be in contact with macrophyte material that contains proportionately lower concentrations of nutrients. The availability of macrophytically derived nutrients to EP was not determined. However, the TP content of EP was not statistically greater than FP, suggesting that macrophyte P was not a significant, addtional P source. Total inorganic C and subsequently $CaCO_3$ contents of the dead material was greater than that of the live material. The accumulation of EP on the dead stems may lead to calcification in the decaying vegetation.

Table 2-3. Characterization of sawgrass (*Cladium jamaicense* Crantz.) stems from within (live and dead) and above water surface collected from WCA-2A-217 on April 10, 1995. Below water stems were substrate for epiphytic periphyton (n = 4 unless noted † then n = 3). Levels of significantly different sample means are given by p-values (***=0.001, **= 0.01, *= 0.05, ns > 0.05). Values followed by similar letters are not significantly different.

			Below Water		Above Water
	Units	p-value	Live	Dead	Live
Parameter	(dry)		Mean ± SE	Mean ± SE	Mean ± SE
Total P	mg kg^{-1}	***	165 ± 20^{a}	28 ± 5	197 ± 15^{a}
Total Ca	g kg^{-1}	ns	24.4 ± 5.5	38.3 ± 5.1	38.9 ± 2.5
Total Mg	g kg^{-1}	**	0.02 ± 0.02^{b}	1.42 ± 0.28^{a}	1.10 ± 0.22^{a}
Total Al	g kg^{-1}	ns	0.10 ± 0.03	0.11 ± 0.01	0.06 ± 0.02
Total Fe	g kg^{-1}	***	$< 0.003^{b}$	0.12 ± 0.02^{a}	0.07 ± 0.01^{a}
Water content	%	***	74.1 ± 1.9^{a}	98.3 ± 0.3^{b}	60.0 ± 2.0^{c}
Ash	%	**	2.67 ± 0.23^{a}	3.57 ± 0.58^{ab}	4.78 ± 0.26^{b}
Total N	g kg^{-1}	**	$3.53 \pm 0.25^{\dagger a}$	$3.68 \pm 0.68^{\dagger a}$	5.71 ± 0.06^{b}
Total C	g kg^{-1}	**	$455 \pm 3^{\dagger a}$	$448 \pm 5^{\dagger ab}$	442 ± 1^{b}
TC\TN		**	130 ± 11	130 ± 23	77 ± 1
Total Inorg. C	g kg^{-1}	***	$0.8 \pm 0.3^{\dagger a}$	$68.0 \pm 5.8^{\dagger b}$	9.5 ± 2.1^{a}
Total Org. C	g kg^{-1}	***	$454 \pm 2^{\dagger a}$	$380 \pm 10^{\dagger b}$	432 ± 3^{a}
$CaCO_3$	g kg^{-1}	***	$6.7 \pm 3^{\dagger a}$	$566 \pm 42^{\dagger b}$	79.0 ± 17^{a}

Phosphorus Forms in Soils and Benthic Periphyton

Soil and BP collected in August 1993 were characterized for P forms and selected cations associated with P retention. Total P in BP equaled that in the surface layer of the soil column (Fig. 2-3g.) (Table 2-4). Total P remained constant to a depth of 4-5 cm then decreased to approximately one-half that of the surface at 15-20cm depth (Fig. 2-3g). Concentrations of all soil P forms generally decreased exponentially with depth. Non-linear regression analysis showed surface layers contained significantly greater P in all fractions than did lower soil layers ($\alpha = 0.05$, r^2 ranged 0.53 - 0.98). This trend was most pronounced for Ca-P_i, Fe/Al-P_i, labile P_o, residual P_o, and TP and was less distinct for labile P_i, and hydrolyzable P_o (Fig. 2-3a-g.). The parameters - labile P_o ($r = 0.57^{***}$), Fe/Al P_i ($r = 0.57^{***}$), Ca-P_i ($r = 0.56^{***}$), residual P_o ($r = 0.64^{***}$) and TP ($r = 0.88^{***}$) - were correlated with depth suggesting higher concentrations at the soil surface. Depth of the BP layer at the time of sampling was 7 cm. Depth values for the BP layer are represented at +3 cm (Fig. 2-3). Of the extracted P forms, labile P_o ($r = 0.78^{***}$), Fe/Al-P_i ($r = 0.66^{***}$), and Ca-P_i ($r = 0.54^{***}$) were most highly correlated to TP.

Labile P forms (P_i and P_o) comprised 12 -18 % of TP (Fig 2-4). Being labile, these forms of P can have the greatest effect on surface water quality by being those most biologically available. Labile P_i accounts for a smaller percentage of TP in the BP than in all soil layers (Fig. 2-4). On a mass basis, labile P_i in BP is also smaller than in all soil layers, even when soil TP drops to half that of the BP (Fig. 2-3a). This agrees with the P limited condition of WCA-2A-217. Any readily available P_i is rapidly incorporated into the periphyton biomass (see Chapter 3). Similarly, the labile P_o of the BP is lower than surficial soils on a mass basis (Fig. 2-3b), but this trend is not as distinct as that of labile P_i. Benthic periphyton was also shown to rapidly incorporate organic P, such as adenosine triphosphate (ATP) (see Chapter 3).

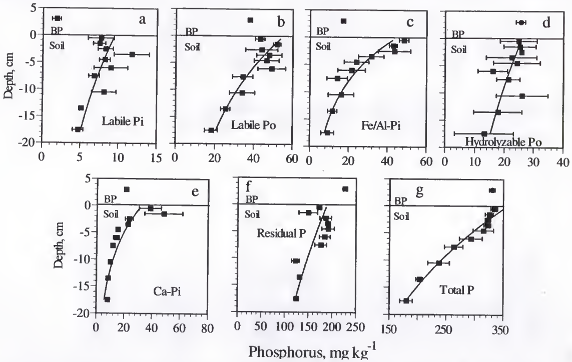

Fig. 2-3. Distribution of P forms extracted from WCA-2A-217 benthic periphyton and soil collected in August, 1993. Depth of BP, averaging 7 cm is graphicaly represented by point at +3 cm. Data are means ± SE of 4 replicate soil cores and 3 BP samples.

Table 2-4. Characterization of WCA-2A-217 benthic periphyton (BP) and soil collected August 12, 1993. (n = 4, if noted † then n = 3).

Depth Increment	Units	BP	0-1cm	1-2cm	2-3cm	3-4cm	4-5cm
	Mean ± SE						
Total P	mg kg^{-1}	330 ± 5	334 ± 6	325 ± 7	323 ± 5	322 ± 6	315 ± 18
1 M HCl extractable P_i	mg kg^{-1}	127.9 ± 2.3†	81.9 ± 4.5	73.2 ± 1.0	70.6 ± 1.0	70.7 ± 2.4	69.2 ± 2.0
1 M HCl extractable Ca	g kg^{-1}	112.9 ± 4.0	48.8 ± 0.9	42.9 ± 2.0	39.5 ± 1.7	37.5 ± 1.3	35.7 ± 0.9
1 M HCl extractable Mg	g kg^{-1}	4.0 ± 0.1	3.4 ± 0.0	3.2 ± 0.1	3.3 ± 0.1	3.1 ± 0.1	3.2 ± 0.1
1 M HCl extractable Al	mg kg^{-1}	305 ± 91	630 ± 20	598 ± 53	553 ± 90	510 ± 78	475 ± 76
1 M HCl extractable Fe	mg kg^{-1}	335 ± 23	223 ± 20	208 ± 30	175 ± 13	185 ± 16	180 ± 16
pH (mean)			7.72	7.64	7.45	7.44	7.34
pH (range)			7.64-7.85	7.49-7.75	7.29-7.73	7.25-7.62	7.21-7.57
Bulk Density	g cm^{-3}	0.011 ±<0.001	0.112 ± 0.005	0.110 ± 0.014	0.093 ± 0.019	0.089 ± 0.014	0.093 ± 0.014
Water Content	%	93.6 ± 0.3†	86.6 ± 1.1	87.3 ± 1.8	87.9 ± 1.9	88.7 ± 1.3	89.2 ± 0.9
Ash	%	33.4 ± 2.1	15.6 ± 0.6	13.7 ± 0.6	12.4 ± 1.9	11.8 ± 0.8	11.1 ± 0.8
Total C	g kg^{-1}	321 ± 38	415 ± 6	426 ± 8	435 ± 8†	441 ± 8	447 ± 6†
Total Inorganic C	g kg^{-1}	26.0 ± 2.2	6.8 ± 0.3	5.6 ± 0.6	4.9 ± 0.5	4.6 ± 0.4	4.3 ± 0.3
Total Organic C	g kg^{-1}	295 ± 37	408 ± 6	421 ± 8	431 ± 8†	436 ± 8	443 ± 6†
$CaCO_3$	g kg^{-1}	216.8 ± 18.7	56.4 ± 2.3	46.8 ± 5.0	40.7 ± 4.3	38.3 ± 3.1	35.9 ± 2.7

Depth Increment	Units	5-7cm	7-9cm	9-12cm	12-15cm	15-20cm
Total P	mg kg^{-1}	293 ± 20	263 ± 16	237 ± 19	203 ± 6	180 ± 11
1 M HCl extractable P_i	mg kg^{-1}	61.2 ± 8.3	49.1 ± 2.5	51.8 ± 5.1	40.5 ± 1.9	35.1 ± 1.3
1 M HCl extractable Ca	g kg^{-1}	33.8 ± 2.6	30.8 ± 3.0	33.6 ± 0.7	32.9 ± 0.4	31.6 ± 0.8
1 M HCl extractable Mg	g kg^{-1}	3.0 ± 0.1	2.8 ± 0.3	2.9 ± 0.1	2.9 ± 0.1	2.7 ± 0.1
1 M HCl extractable Al	mg kg^{-1}	465 ± 99	375 ± 89	483 ± 60	548 ± 51	575 ± 29
1 M HCl extractable Fe	mg kg^{-1}	160 ± 14	178 ± 43	213 ± 22	230 ± 18	230 ± 19
pH (mean)		7.22	7.22	7.15	7.39	7.27
pH (range)		6.96-7.40	7.07-7.43	6.88-7.39	7.29-7.44	7.00-7.55
Bulk Density	g cm^{-3}	0.071 ± 0.011	0.077 ± 0.007	0.075 ± 0.006	0.083 ± 0.004	0.084 ± 0.003
Soil Water	%	89.6 ± 0.7	90.1 ± 0.5	90.4 ± 0.3	90.2 ± 0.1	90.4 ± 0.3
Ash	%	10.7 ± 0.8	9.9 ± 0.5	9.4 ± 0.2	9.3 ± 0.3	9.0 ± 0.2
Total C	g kg^{-1}	427 ± 17	457 ± 5	467 ± 5	467 ± 10	456 ± 7
Total Inorganic C	g kg^{-1}	4.1 ± 0.3	4.2 ± 0.3	4.3 ± 0.4	4.1 ± 0.3	4.1 ± 0.2
Total Organic C	g kg^{-1}	424 ± 17	457 ± 5	463 ± 5	463 ± 10	452 ± 8
$CaCO_3$	g kg^{-1}	34.5 ± 2.7	35.3 ± 2.5	35.6 ± 3.0	34.2 ± 2.3	34.0 ± 1.7

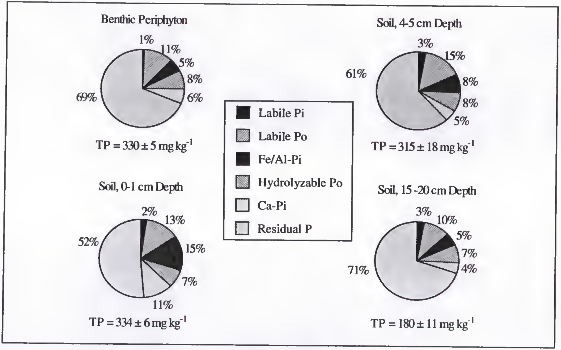

Fig. 2-4. Average relative extracted P form as a percentage of total P (TP) in benthic periphyton (BP) and selected soil depths. Total P content by perchloric acid digestion is shown under diagrams.

The Fe/Al-bound P_i makes up 15 % of TP in the surface 0-1 cm of the soil and decreased depth to 4% of TP. Hydrolyzable P_o maintains a consistent percentage of TP, accounting for about 7 - 8 % in all depths (Fig. 2-4). However, the extracted amounts of this fraction were highly variable (Fig. 2-3d). The Fe/Al-bound P_i fraction may be overestimated. The NaOH extractant used to separate this fraction solubilizes humic and fulvic acids and therefore may be releasing some organically bound P. Further evidence to suspect overestimation of Fe/Al-P_i is that the extracted solutions analyzed were highly colored, and the 1 *M* HCl-extractable concentrations of Fe and Al were relatively low compared to the amount of P_i. This portion of the sequential extraction was suspect in other studies (Koch and Reddy, 1992). Calcium bound P_i accounted for between 4 and 11 % of TP. There is a very distinct enrichment of this fraction in the surface 0-2 cm of soil depth (Fig. 2-3e).

The residual P accounted for the largest portion of P in all samples (Figs. 2-3f and 2-4). Residual P as a percentage of total extractable P was similar in the BP and the 15 - 20 cm soil depths (Fig. 2-4). For this organic soil, the residual P is probably composed mostly of recalcitrant organic material and not resistant mineral forms. The residual P, also known as recalcitrant organic P, was found to be the fraction in largest proportion in other studies on Everglades peat soil (Reddy et al., 1997; Qualls and Richardson, 1995). The decrease in residual P between the BP layer and that of the surficial soils and a corresponding increase in the inorganic fractions (labile P_i, Fe/Al- P_i, Ca-P_i) is evidence of increased mineralization in surficial soils. Increased biological activity in this layer probably increased mineralization of decomposing BP. The residual P fraction increased as a proportion of TP after the first few surficial depths because the remaining material becomes increasingly resistant to microbial degradation. Phosphorus mineralization in peat soils of fens in the Netherlands was shown to be faster at a soil depth of 10 cm than at 25 cm (Verhoeven et al., 1990). Verhoeven and coworkers (1990) believed the increased mineralization was due to the effects of temperature and/or redox conditions on microbial metabolic activity but also suggest that a change in substrate quality with depth could have been partially responsible.

In addition to the sequential P fractionation, soils and BP samples were analyzed for several physicochemical parameters (Table 2-4). Soil bulk density ranged 0.07 - 0.11 g cm^{-3} and generally decreased with depth, although the correlation was weak ($r = 0.35^{*}$). The bulk density of these soils were lower than those of the Netherlands minerotrophic fens, 0.07 - 0.25 g cm^{-3} (Verhoeven, 1986), but agrees well with those from several other locations in the Everglades (Koch and Reddy, 1992; Craft and Richardson, 1993) The pH of the soils averaged slightly above neutral with the highest recorded pH (7.85) being in the 0 - 1 cm depth increment and the lowest (6.88) being at 9-12 cm. Ash ($r = 0.68^{***}$), $CaCO_3$ ($r = 0.57^{***}$), and TIC ($r = 0.57^{***}$) contents were correlated to depth. The $CaCO_3$ content of the BP averaged almost 22% (dry wt. basis). High $CaCO_3$ content are expected in the calcareous cyanobacteria dominated BP of WCA-2A-217 (Swift and Nicholas, 1987). The $CaCO_3$ content of the BP during this sampling was lower than was seen at this site during the April 1995 sampling (Table 2-2). Conversely, there was an inverse relationship between TC and TOC with proximity to the soil surface. Total C and TOC concentrations increased with depth in the soil column.

Phosphorus and selected cation concentrations were analyzed after extraction of air dried, ground soil samples with 1 *M* HCl. The P_i extracted with 1 *M* HCl also estimates total soil P_i (TP_i). The TP_i contents were slightly higher than that estimated by sum of the sequential extraction. Both methods showed similar trends with depth. Recently, Reddy et al., (1997) analyzed TP_i by sequential and 1 *M* HCl extraction for 210 soil samples collected from a large area of the Everglades. They showed that the empirical relationship between the two estimates closely agreed. The TP_i was strongly correlated to HCl extracted Ca ($r = 0.87^{***}$), and Mg ($r = 0.79^{***}$), was weakly correlated to extracted Fe ($r = 0.44^{***}$), and was not correlated to extracted Al ($r = -0.24^{ns}$). Total P_i was strongly correlated with depth ($r = 0.87^{***}$). Extracted cations were generally correlated with ash content except for Al. The concentrations of extracted Ca in the soil column were 55 - 85 times higher than extracted Al and between 130 and 230 times greater than extracted Fe. Extracted Mg averaged 7 and 15 times greater than soil Al and Fe, respectively (Table 2-4). The extractable Ca was correlated with depth (r = 0.64***) showing an accumulation of Ca in the soil surface. Phosphorus complexed with cations in the soil is dominated by the Ca/Mg cations, not Al/Fe. This is consistent with the alkaline nature of the site. Aluminum and Fe tend to dominate P complexation in acid soils. The relatively low concentrations of Fe in WCA-2A-217 soil suggests that P retention would not be strongly influenced by redox conditions.

A value termed the inorganic P index (IPI) was developed to describe changes in the proportion of total P that was composed of by P_i. The IPI was determined by summing the inorganic P forms and expressing the total as a ratio of TP

$$IPI = (\text{labile } P_i + \text{Fe/Al-}P_i + \text{Ca-}P_i) / TP \qquad [2\text{-}3]$$

The IPI for WCA-2A-217 soils was larger in the surface 0 - 4 cm (Fig 2-5) and decreased exponentially with depth. A nonlinear regression analysis of the relationship of IPI to depth showed IPI to be statistically higher in the surface soil than with depth ($\alpha = 0.05$). The equation

$$IPI = 0.216\, e^{-0.335\, x} + 0.130 \qquad [2\text{-}4]$$

where x = soil depth, best described the trend (r2 = 0.64). The relative contribution of P_o forms to TP content increased with depth. This, coupled with increased levels of $CaCO_3$, HCl-extractable P_i, Ca, and Mg in the soil surface suggests accumulation of P_i in the surface soils. It is hypothesized that accumulation of P_i forms in the surface soil is due to the decomposition of organic materials, which results in the release of labile P_o that is then mineralized to P_i. Some of the P_i is then recycled back into the biota while a portion is precipitated with Ca, coprecipitated with $CaCO_3$, or otherwise absorbed to

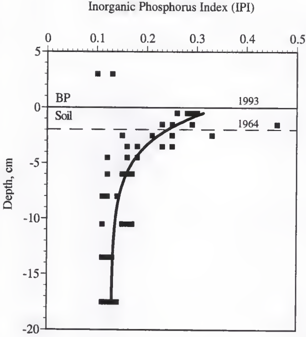

Fig. 2-5. Inorganic phosphorus index (IPI) with depth in the soil column. Inorganic P index is defined as the ratio of all extracted inorganic P forms to total P. IPI = (labile P_i + Fe/Al-P_i + Ca-P_i) / (TP). Soil/benthic periphyton (BP) interface is shown for 1993 by solid line and by dashed line for soil/BP interface position in 1964 as determined by ^{137}Cs dating.

inorganic solid phases. The exact mechanism, and the relative proportions of P_i being recycled versus precipitated on a short-term basis are currently unknown (Reddy et al., 1997).

Data from the sequential extraction of the 1993 soils were averaged over the 0-2 and 2-5 cm increments so that comparisons could be made between soil chemistry of August 1993 and April 1995. Statistical comparisons were made for each of the two depth increments. Significant comparisons are denoted by p-values (Table 2-5). Although not statistically different the bulk density of the 0-2 cm increment was higher in the 1993 soils than in the 1995 soils. For the 2-5 cm increment, the bulk densities are the same; therefore, concentration data are directly comparable. Concentrations of P in 1993 surface soils were slightly lower than in 1995 when normalized for bulk densities. There was generally little difference in labile P concentrations in the 2-5 cm soils from the two sampling dates; however, the TP concentrations in the 2 - 5 cm soil was higher in 1995 than in 1993. The TP data for the 1995 sampling was more variable than that of the 1993 samples. Although statistically significant, the differences in TP concentration may be due as much to spatial variation as to temporal P changes. The 1 *M* HCl extracted Ca, and ash contents of the 1993 soils were significantly lower than those of 1995. This indicates a lower degree of calcification in the 1993 soils. Conversely, this difference was not seen in the $CaCO_3$ or TIC contents, as expected if there was a difference in mineralogical components of the soils. Differences in the pH of the soils between sampling times did not manifest in changes in the $CaCO_3$ contents suggesting that the $CaCO_3$ is stable at pH slightly lower than neutral. Overall, the soil samples collected 21 months apart were not appreciably different, attesting to the stable nature of the interior sites of WCA-2A.

Table 2-5. Characterization of WCA-2A-217 surface soils collected August 1993 and April 1995 (n = 4). Statistical comparisons were made between sampling times for soil parameters of each depth increment. (p-values: *** = 0.001,** = 0.01, * = 0.05, ns > 0.05).

		August 1993			April 1995		
Soil Depth Parameter	Units (dry wt.)	0-2cm Mean ± SE	2-5cm Mean ± SE	0-pval.	0-2cm Mean ± SE	2-5cm Mean ± SE	2-pval.
Labile P_i	mg kg^{-1}	7.6 ± 0.8	9.4 ± 0.9	**	13.0 ± 0.5	16.2 ± 3.5	ns
Labile P_o	mg kg^{-1}	47.5 ± 2.2	46.1 ± 3.8	*	104.8 ± 20.1	77.5 ± 15.0	ns
Total P	mg kg^{-1}	298 ± 10	253 ± 8	*	427 ± 45	403 ± 46	*
1 *M* HCl							
P_i	mg kg^{-1}	78 ± 3	70 ± 1	ns	148 ± 32	124 ± 33	ns
Ca	g kg^{-1}	45.9 ± 1.5	37.6 ± 0.8	***	78.1 ± 2.4	62.9 ± 2.0	***
Mg	g kg^{-1}	3.29 ± 0.06	3.22 ± 0.06	**	3.48 ± 0.03	3.30 ± 0.06	ns
Al	g kg^{-1}	0.61 ± 0.03	0.51 ± 0.04	ns	0.48 ± 0.05	0.52 ± 0.05	ns
Fe	g kg^{-1}	0.22 ± 0.02	0.18 ± 0.01	ns	0.20 ± 0.02	0.19 ± 0.01	ns
Total Ca	g kg^{-1}	NA			117 ± 4	93 ± 4	
Total Mg	g kg^{-1}	NA			5.47 ± 0.26	5.48 ± 0.26	
Total Al	g kg^{-1}	NA			2.12 ± 0.11	2.09 ± 0.17	
Total Fe	g kg^{-1}	NA			1.72 ± 0.11	1.58 ± 0.16	
pH (mean)		7.7	7.4	**	7.0	6.9	**
pH (range)		7.5-7.9	7.2-7.7		6.7-7.3	6.7-7.1	
Bulk Density	g cm^{-3}	0.11 ± 0.01	0.09 ± 0.01	ns	0.08 ± 0.01	0.09 ± 0.01	ns
Water Content	%	86.9 ± 1.0	88.6 ± 0.8	*	91.2 ± 0.5	90.4 ± 0.9	ns
Ash	%	14.7 ± 0.5	11.7 ± 0.5	*	17.5 ± 0.5	14.7 ± 0.5	*
Total N	g kg^{-1}	NA			31.4 ± 0.8	33.0 ± 0.7	ns
Total C	g kg^{-1}	421 ± 5	442 ± 4	ns	430 ± 4	455 ± 6	ns
TC:TN		NA			13.7 ± 0.4	13.8 ± 0.5	
Total in. C	g kg^{-1}	6.2 ± 0.4	4.6 ± 0.2	ns	4.9 ± 0.6	5.5 ± 1.1	ns
Total org. C	g kg^{-1}	414 ± 5	437 ± 4	ns	425 ± 4	449 ± 6	ns
$CaCO_3$	g kg^{-1}	51.6 ± 3.1	38.3 ± 1.9	ns	40.5 ± 5.2	45.7 ± 9.2	ns

NA = not available

Long-term Peat and Nutrient Accretion Rates

The peak ^{137}Cs concentration occurred at 2 cm soil depth (Fig. 2-6) which represents a peat accretion rate of 0.68 mm yr^{-1}. This level of peat accretion was lower than the 4 - 11.3 mm yr^{-1} accretion rates found by Reddy et al., (1993) for a transect along a nutrient gradient in WCA-2A. Craft and Richardson (1993) found accretion rates in unenriched WCA-2A to average 1.6 mm yr^{-1}. Several factors influence the peat accretion rates including hydrology and the level of nutrients. For instance, Reddy et al., (1993) found decreasing peat accretion rates with increasing distance from nutrient sources in WCA-2A. Hydrology affects peat accretion by decreasing the oxidation of organic matter under increased water depth and duration. The water depth at WCA-2A-217 periodically drops below the soil surface (SFWMD, data not shown) allowing oxidation of the peat layers. Accumulation rates of P, C, and selected cations since 1964 were calculated for 4 replicate cores (Table 2-6). The accretion rates of organic P

forms: labile P_o, Hydrolyzable P_o, and residual P_o, are about 2.5 times higher than that of the inorganic P forms; Labile P_i, Fe/Al-bound P_i, and Ca-bound P_i. Organic P forms accounted for 71%, and inorganic P forms made up 29% of the TP accumulated since 1964. Accumulation rates are dependent upon two factors: accretion of peat and concentration of materials in the peat (Qualls and Richardson, 1995).

Estimated accretion rates and the standing TP content in BP biomass at the time of sampling were used to calculate the proportion of BP-TP incorporated into the surface soil. On an areal basis, the BP contained 254 mg m^{-2} and the TP accumulation in the surface 2 cm of soil was 25.2 mg m^{-2} yr^{-1} over the past 29 yrs. Dividing accumulated TP by that in the standing BP results in approximately 10% of the TP in the BP accumulating in the surface soil. These calculations assume a constant standing stock of BP, constant TP content in the BP, and the same accumulation rate in each of the past 29 yrs. Under the caveat of these assumptions, it is hypothesized that 10% of the P content of BP is

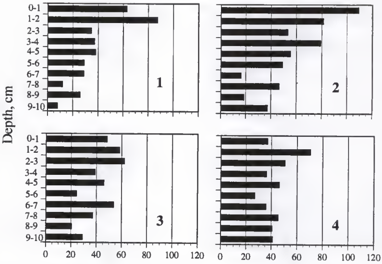

Fig. 2-6. Depth distribution of ^{137}Cs in four replicate soil cores of WCA-2A-217 collected in August 1993. Maximum ^{137}Cs accumulation denotes soil surface in 1964. Maximum generally occurs within 2 cm of soil surface.

Table 2-6. Peat accretion rate and accumulation rate of P forms and selected parameters since 1964. Based on depth to ^{137}Cs peak at 2 cm in soil profiles of four replicate cores.

Parameter	Accumulation Rate (Mean ± SE, n = 4)	Accumulation as Percentage of Total P
Peat	0.7 mm yr^{-1}	
	mg m^{-2} yr^{-1}	
Labile P_i	0.6 ± 0.1	2.3 ± 0.4
Labile P_o	3.6 ± 0.3	14.4 ± 0.5
Fe/Al-bound P_i	3.4 ± 0.4	13.9 ± 1.9
Hydrolyzable P_o	1.9 ± 0.5	7.5 ± 1.6
Ca-bound P_i	3.3 ± 0.6	13.0 ± 2.2
Residual P	12.3 ± 0.8	48.9 ± 2.6
Total P	25.2 ± 1.7	
HCl Extractable		
P_i	5.9 ± 0.4	23.5 ± 0.7
Mg	251 ± 12	
Al	47.5 ± 5.0	
Fe	16.6 ± 2.0	
	g m^{-2} yr^{-1}	
Ca	3.5 ± 0.1	
Total Carbon	32.1 ± 1.5	
Total Inorganic C	0.5 ± 0.0	
Total Organic C	31.6 ± 1.5	
$CaCO_3$	3.9 ± 0.1	

added to the soil each year; 90% is either recycled through the BP or exported downstream. Similarly, estimates of TC accretion result in 13% of the TC in the BP being incorporated into the soil.

Conclusions

The interior of WCA-2A, as exemplified by site 217, can be characterized as an oligotrophic, minerotrophic peatland. The TP content of the water was around 10 $\mu g\ L^{-1}$ with DRP accounting for 90% of TP. The DRP:TP is evidence of a P-limited system with mechanisms developed to maximize P availability. Calcareous cyanobacterial periphyton exists as floating, epiphytic, and benthic forms. Phosphorus contents of these types are in the order; BP > EP = FP. The higher TP contents of the BP reflect the water column position. Forming a layer between the floodwater and the soil surface the BP receives detrital materials from the water column. Epiphytic periphyton preferentially colonized dead sawgrass stems. Sawgrass stems probably provide only physical support as the P concentration in the dead material was very low. However, the exchange of nutrients between the macrophyte and EP was not studied.

The peat soil contained TP concentrations around 330 mg kg^{-1} in the surface and about half that at 15-20 cm. Similarly, the concentrations of all P forms generally decreased exponentially with depth. Residual P, considered to be recalcitrant organic P, accounted for the largest fraction of TP. Inorganic P was complexed with Ca and Mg reflecting the alkaline nature of the site. The inorganic P index (IPI) showed proportionally higher P_i at the soil surface than at depth, suggesting increased complexation with Ca or other inorganic solid phases at the soil surface.

Peat accretion, based on ^{137}Cs dating, is slow, accounting for up to 0.7 mm yr^{-1}. Estimates of BP-TP entering the soil are about 10% yr^{-1}. Stage recording shows periodic drying of this area, thus allowing oxidation. This reduces net accretion.

CHAPTER 3
PHOSPHORUS UPTAKE AND PARTITIONING BY PERIPHYTON

Introduction

Phosphorus is an essential nutrient that frequently limits the productivity of freshwater ecosystems. The cycling of phosphorus (P) in wetlands involves numerous interactions between the P compartments of the water, biota, and soil. Processes controlling these interactions can be biological, as in uptake and release by periphyton and vegetation or physicochemical, such as adsorption/precipitation. Periphyton refers to the communities of attached microorganisms, both floral and faunal, that grow on submerged surfaces (Wetzel, 1983). Periphyton, often a 2conspicuous feature of shallow water bodies, may contribute up to 80% of the primary productivity to an aquatic system and can therefore influence many of the biological and physicochemical aspects of these environments (Hansson, 1992; Carlton and Wetzel, 1988).

Periphyton covers a large extent of the oligotrophic Everglades Marsh. It is believed that the activity of calcareous periphyton has had a primary role in the biogenic formation of the calcitic mud found in the southern Everglades (Gleason, 1972). The calcitic nature of the surface soil and periphyton plays a significant role in the long-term P storage (Reddy et al., 1993). The uptake of P by periphyton is dependent upon P concentration in the water column, antecedent P content in the periphyton tissue, the forms of P available, and the growth stage and thickness of the mat (Sand-Jensen, 1983; Horner et al., 1983; Cotner and Wetzel, 1992). Several studies suggest that algal tissue P content in the range of 1.1 - 2.8 mg g^{-1} (dry wt.) is necessary for normal growth (Müller, 1983; Riber et al., 1983; Swift and Nicholas, 1987). However, Grimshaw et al. (1993) observed that the P content of periphytic material in oligotrophic areas of the Everglades Water Conservation Areas (WCA) was <0.1 mg g^{-1}. Generally, as dissolved reactive P (DRP) in water increases, so does the cellular P content of the periphyton (Swift and Nicholas, 1987; Adey et al., 1993). Many species of algae have been found to accumulate P at concentrations much higher than needed for normal growth. This "luxury uptake" has been shown in numerous studies with enriched waters (Müller, 1983; Riber et al., 1983; Swift and Nicholas, 1987; Cotner and Wetzel, 1992). In a recent study, Adey and coworkers (1993) found that the P content in periphyton, cultured in agricultural drainage water, increased to a saturation level of approximately 3.5 - 4.2 mg g^{-1} (dry wt.) at solution P concentrations as low as 25 $\mu g\ L^{-1}$. However, it should be noted that P saturation is dictated by P loading (mass of P applied per unit biomass or area) not by concentration alone.

In addition to the uptake of inorganic P (P_i), numerous species of algae are able to utilize organic P (P_o) (Cotner and Wetzel, 1992; Bentzen et al., 1992). A number of factors, including C:P ratio of organic matter undergoing decomposition and rate of phosphatase enzyme production, are important in regulating organic P mineralization (Golterman, 1973; Syers et al., 1973; Newman and Reddy, 1993). Bentzen and coworkers (1992) found a significant effect of dissolved P_i concentration on P_o uptake by limnetic plankton, but concluded that dissolved P_o was also actively utilized when demand was high.

The calcareous cyanobacteria that dominate the periphyton in wetlands such as the interior of WCA-2A are known to cause the precipitation of $CaCO_3$ (Gleason, 1972). This $CaCO_3$ is commonly seen as sheaths, stalks, or other encrustations. It is hypothesized that P can be complexed with this $CaCO_3$ leading to abiotic removal of P from the water column. Recently, Browder et al. (1994) posed the premise that P adsorption to $CaCO_3$ may be controlling watercolumn P levels. In this context, periphyton removal of water column P column would then be a function of both abiotic and biotic processes.

Previous research on the periphyton in the WCAs (Swift, 1981; Swift and Nicholas, 1987) focused on how environmental variables such as light, hydroperiod, and water quality factors affect the growth and species composition of the mats. However, limited information is available on the influence of periphytic activity on the fate of P in the water column.

The objectives of this study were to determine the P_i and P_o uptake kinetics of periphyton, compare P_i uptake kinetics between morphologically different periphyton types, and to assess the relative magnitude of biotic and abiotic uptake mechanisms. Phosphorus uptake was determined in a series of laboratory and field studies using a solution depletion technique (Reuter et al., 1986). Partitioning of periphyton incorporated P by biotic and abiotic mechanisms was evaluated using ^{32}P and a simple extraction procedure.

Materials and Methods

Study Site

The original Everglades has been fragmented into a series of hydrologic units consisting of the Everglades Agricultural Area (EAA), three Water Conservation Areas (WCA) and Everglades National Park (ENP). The three WCAs encompass almost 3500 km^2, representing a little more than one third of the original Everglades (Swift and Nicholas, 1987). Water Conservation Area 2 is the smallest of the three WCAs (547 km^2), lies between WCA-1 and WCA-3A, and is heavily impacted by large quantities of nutrient enriched water derived from the Everglades Agricultural Areas (EAA) (Swift and Nicholas, 1987). The surface water quality (SWIM, 1992), nutrient gradient (Koch and

Reddy, 1992), and soil nutrient characteristics (DeBusk et al., 1994) of this area have been recently studied. The interior unimpacted sites of WCA-2A are dominated by calcareous blue-green algae (cyanobacteria), such as *Schizothrix calcicola* (Ag.) Gom. and *Scytonema hofmannii* Ag., as well as hardwater diatoms (Swift and Nicholas, 1987). The average periphyton N to P ratio in the impacted sites was 9:1 while at the interior sites this ratio was 107:1, suggesting that the interior of WCA-2A is P limited (Swift and Nicholas, 1987).

Characterization of Periphyton

Periphyton used in laboratory studies was collected from the field site station 217 (Fig. 3-1; 26° 17.17' N, 80° 24.73' W ± 30 m) on October 18, 1994 for initial studies, then again on June 8, 1995 for ^{32}P laboratory studies. Benthic periphyton (BP) was collected by gently running a swimming pool screen of 1 mm mesh size along the soil surface. Epiphytic periphyton (EP) was obtained by clipping senescent stems of sawgrass (*Cladium jamaicense* Crantz.) just above the soil surface. Periphyton, forming a nearly continuous coating on soils and sawgrass and extending into the water as floating material, was collected, gently homogenized, and termed mixed periphyton (MP). Upon return to the laboratory EP was removed from the stems by scrapping with a scalpel. Periphyton was placed in plastic trays filled with bulk surface water, also field collected, and stored with occasional stirring in a light booth that provided 10 W m^{-2} on a 16 h light/8 h dark cycle. Subsamples were removed as needed for experiments.

Portions of the bulk periphyton material were oven dried at 70° C to constant weight. A known mass of dry periphyton was combusted in a muffle furnace at 450° C for 12 h to determine ash content. Subsamples were digested with perchloric acid (4500-P-B, APHA, 1992) and the digestate was analyzed colorimetrically for total P (TP) (Method 4500-P-F, APHA, 1992). Total C (TC) and N (TN) were determined on oven-

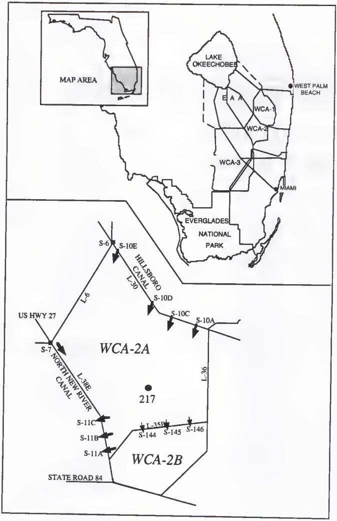

Fig. 3-1. Map of Water Conservation Area 2A (WCA) showing field site 217.

dried samples using a Carlo-Erba NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, New Jersey) (Nelson and Sommers, 1982). Total inorganic C (TIC) was determined on ashed material, as above, using the CNS analyzer. Total organic C (TOC) was determined by difference (Browder et al., 1982).

In addition, BP collected in 4 replicate bulk samples on June 8, 1995 was also characterized for bulk density and $CaCO_3$-associated P ($CaCO_3$-P). Bulk density was determined by collecting a known volume of BP in an acrylic cylinder. Measurements were made to insure negligible compaction and to provide depths of the BP layers. The $CaCO_3$-P of fresh BP samples was extracted in a 1:100 solid to solution ratio with 0.01 *M* HCl for 2 h (see following section). Extractant solution was filtered (0.2 µm, Gelman Supor, Gelman Inc., or Cameo acetate membrane, MSI Inc.) and analyzed colorimetrically for DRP on an AutoAnalyzer II (Technicon Corp.) (Method 4500-P-F, APHA, 1992).

Water samples were collected and filtered (0.2 µm) in the field on June 7 and 8, 1995. Samples were analyzed for DRP and digested by persulfate oxidation (Method 4500-P-B, APHA, 1992) and analyzed for total dissolved P (TDP) (Method 4500-P-F, APHA, 1992).

Development of Extraction Procedures

It was hypothesized that the P abiotically associated with periphyton would be bound to $CaCO_3$ ($CaCO_3$-P). Preliminary analysis of dried, ground BP resulted in a $CaCO_3$ content of about 50%. Removing the $CaCO_3$ by dissolution would cause the release of this P. An optimal extractant would remove $CaCO_3$-P but would not remove appreciable amounts of biotically bound organic P. Assuming the extractant removes all $CaCO_3$-P and that extracted solution TDP = DRP then either no biotic P was extracted or any biotic P_o extracted was completely hydrolyzed. A series of preliminary experiments were conducted using varying molarities of HCl, solid:solution ratios, and extractant times. Fresh BP was gently homogenized and mixed with the acid extractant. Samples were collected at selected times, 0.2 μm filtered, and analyzed colorimetrically for DRP. A portion of this subsample was digested by persulfate oxidation and analyzed colorimetrically for TDP. The results of these tests showed that 0.01 *M* HCl in an approximately 1:100 fresh weight BP to solution ratio provided highest extracted P concentrations yet maintained the relationship TDP = DRP. At weaker molarities very little P was released, and at higher molarities TDP > DRP.

In the first of the experiments presented which demonstrate the applicability of this method, duplicate fresh BP samples were extracted with 0.01 *M* HCl (1:100). Solution samples were collected at 2, 5, 10, 30, 60, and 120 minutes, 0.2 μm filtered and analyzed colorimetrically for DRP. An aliquot of this sample was analyzed for TDP. Differences between the DRP and TDP concentrations in the extract were analyzed for significance by t-tests. In the course of continuing experimentation, additional BP samples were collected and subjected to a 2 h extraction with 0.01 *M* HCl. These data were also used to substantiate the method.

The pH of the BP containing extracting solution was generally 2.5 - 3.0. To determine if this level of acidity could hydrolyze soluble organic P, selected inorganic and organic P compounds were added to a known amount of $CaCO_3$ and subsequently extracted with 0.01 *M* HCl. Reagent grade KH_2PO_4 was the source of P_i. Both glucose 6-phosphate (G6P) and ATP were used as P_o. Additions of these compounds were combined with a mass of $CaCO_3$ typically found in periphyton, about 0.5g g^{-1} dry weight, and allowed to equilibrate in a dessicator for about 16 h before extraction. All P containing species were added in amounts to increase the P content per volume of extractant solution by 0.9 μM. The 0.01 *M* HCl was added to these samples and gently stirred periodically for the next 2 h. The pH was monitored during the extraction.

Samples were collected at 10, 30, 60, and 120 minutes. Samples were 0.2 µm filtered and analyzed colorimetrically for DRP or were digested and analyzed for TDP.

Benthic periphyton was examined under a scanning electron microscope (SEM) to confirm the removal of $CaCO_3$ after acid extraction. One subsample of BP was extracted with 0.01 *M* HCl as above; the other was not acid treated. Samples were fixed in Trumps solution, washed in phosphate buffer saline, dehydrated by an ethanol series and dried via hexamethyldisilazane (HMDS) (Nation, 1983). After evaporation to dryness in a dessicator, the samples were attached to carbon coated posts with graphite glue. The posts were then sputter coated with gold and observed on a field emission SEM (S-4000, Hitachi, Tokyo, Japan).

Phosphorus Uptake by Periphyton: Laboratory Conditions.

On October 20, 1994, laboratory experiments began with the incubation of approximately 2 g periphyton with 200 ml bulk water (obtained from the field site) to achieve a nominal ratio of 1:100 freshweight to solution. Each of the three types of periphyton: EP, MP, and BP, were gently homogenized before incubation. Inorganic P as KH_2PO_4 was added in 2 ml aliquots of 10 mg L^{-1} stock solution to bring the initial concentration to 100 g L^{-1} above ambient (Co = 4.7 -6.0 µ*M*, normalized for periphyton dry weight). Triplicate vessels of each periphytic type and a control without added periphyton were incubated under the light booth with occasional stirring. Temperature was approximately 27° C, and light intensity was 10 W m^{-2}. Solution samples (10 ml) were collected at 0, 5, 15, 30, and 45 min after spiking, filtered through 0.2 µm, and analyzed colorimetrically for DRP by standard methods (see above) on an Alpkem Rapid Flow Analyzer (Perstorp Analytical, Wilsonville, OR). Each sample withdrawal reduced the solution volume originally at 200 ml by 10 ml; therefore, calculations of uptake were corrected for decreasing sample volume. Concentration of DRP in solution was normalized for solution volume and periphyton oven dry weight.

Between October 24 and 26, 1994, a second experiment was conducted with three types of periphyton where each type was incubated for a short time period (maximum incubation period of 90 min) at 15 initial DRP concentrations. Initial DRP , as KH_2PO_4, concentrations ranged from ambient (0.19 μM) to 62.2 μM. Experimental protocol and sample handling was done as above, however, each reaction vessel was sampled only once after a predetermined time. Sampling periods were based on the linear portions of DRP depletion from previous experiments.

On November 23 and 24, 1994, a third experiment was conducted to determine P uptake by two types of periphyton (EP and BP) at several initial DRP concentrations in the range of ambient (0.23 μM) to about 14.5 μM. These experiments were conducted to determine the uptake of P_i by periphyton at numerous initial concentrations in a more narrow concentration range than was provided in previous experiments. Solution samples were collected with time at 10, 60, 120, 240, and 480 min after DRP addition. Experimental methods and analysis were similar to those previously described.

The concentration of all solution samples were corrected for matrix effect, probably Si, which caused slightly elevated levels of DRP when measured colorimetrically on an Alpkem Rapid Flow Analyzer with a 3 cm flowcell. The correction factor was obtained by making additions of standards to previously analyzed samples, analyzing the spiked samples, and back calculating the concentration in the original sample. The calculated concentration was subtracted from the initial analyzed concentration and the difference was obtained. This difference was found to be 0.27 ± 0.01 μM ($n = 8$). Spiked recoveries of samples analyzed by a Technicon AutoAnalyzer II showed no matrix effect; thus, in subsequent experiments this instrument was used for DRP analysis.

Phosphorus Uptake by Periphyton: Field Conditions

On June 8, 1995, a field experiment was conducted to determine the uptake of P_i (as KH_2PO_4) by BP. Bulk surface water was collected in large polycarbonate carboys in the vicinity of WCA-2A-217 on each day of field experimentation. Water depth was 42.3 ± 0.3 cm (mean ± SE, n=4) and BP depth was 12.8 ± 0.5 cm (n=4). Bulk density of the BP layer was 0.015 ± 0.001 g cm^{-3}. A known amount of surface water (900 ml) was added to 946 ml polypropylene mason jars that were fit into floating racks. Styrofoam floats were glued to acrylic sheets to provide flotation and stability. The reaction vessels were open to the atmosphere with the liquid portion almost completely submerged in the water column to help maintain ambient conditions. Approximately 9 g of homogenized BP was added to reaction vessels (jars) to provide a nominal 1:100 fresh-weight BP to water ratio. The water was spiked to achieve five initial concentrations. There were four replications of each concentration. Initial concentrations were ambient (0.19 μM), 1.5, 2.7, 5.6, and 10.6 μM DRP. Samples were collected with time at 5, 15, 30, 45, and 60 minutes after P addition. The water samples (20 ml) were filtered (0.2 µm), stored in ice water, and transported to the laboratory. At the end of the last sampling, the BP in each of the reaction vessels was filtered by gravity onto Whatman #41 filter paper (Whatman, Inc.) and dried at 70° C to constant weight (about 72 h). Since solution volume was reduced by 20 ml with each sample, solution mass P was adjusted accordingly. Phosphorus removed from solution was normalized per gram dry weight of BP.

To determine of hydrolysis and uptake of P_o by BP, an experiment was conducted on June 7, 1995. Experimental methods were similar to those described for the P_i uptake study. Each vessel was spiked with a known volume, (up to 4 ml) of solution containing 1.5 mM P as adenosine triphosphate (ATP), to obtain TDP concentrations of ambient (0.9 μM), 3.3, 5.5, and 10.1 μM. Each treatment was replicated four times. Water samples were collected from each vessel before P addition and at 15, 30, 60, 120, and 240 minutes after spiking. Samples were then filtered and analyzed for DRP and for TDP (APHA, 1992). Benthic periphyton was collected after the uptake study and dried as above. Phosphorus in solution was determined colorimetrically on an AutoAnalyzer II (Technicon Corp.) by standard methods (APHA, 1992).

An unfiltered water control (without BP), spiked to ambient + 3.23 μM P, was included in both experiments. Hydrolysis rates in a periphyton-less water column could be determined by this control during the P_o experiments, and a decrease in the DRP of the control would aid in quantifying the magnitude of chemical precipitation for both experiments.

Calculation of Uptake Rates

The solution DRP concentrations at each sampling time (t) were normalized for solution volume and dry weight periphyton. Dissolved reactive P remaining in solution was plotted against sampling time to obtain depletion curves. Phosphorus uptake was described by two methods:(i) initial uptake rate in the linear portion of depletion curves, and (ii) first-order kinetics for the determination of uptake rate constants.

Initial uptake rate calculations made over a range of initial P concentrations, were used to obtain the parameters needed for the Michaelis-Menten equation:

$$v = (V_{max}\, C)/(K_m + C) \qquad [3\text{-}1]$$

where v = P uptake rate by periphyton (μmol g^{-1} min^{-1}), V_{max} is the maximum P uptake rate by periphyton (μmol g^{-1} min^{-1}), K_m is the concentration of P at one-half V_{max} (μM), and C is the P concentration in solution (μM). All uptake rates were calculated on periphyton dry weight basis.

Maximum uptake rates occur when the P concentration available to periphyton is non-limiting. At these concentrations $C >> K_m$, and equation 3-1 reduces to

$$v = V_{max} \qquad [3\text{-}2]$$

where initial uptake rate equals the maximum uptake rate. Initial uptake rates were calculated from the linear portion of the P depletion curve, which occurred anywhere between 5 and 90 minutes (time in minutes between $t = 0$ and $t = t_x$) after spiking depending on initial P concentration and experimental conditions. Over this range the velocity is constant and independent of C. The initial uptake rates were used to develop Lineweaver-Burk plots. The Lineweaver-Burk plot is a double reciprocal plot which transforms the typical Michaelis-Menten equation into a linear form

$$1/v = (1/Vmax) + (Km/Vmax)(1/C) \qquad [3\text{-}3]$$

In the Lineweaver-Burk plot, the reciprocal of the solution concentration (1/C) at any sampling time is the x-axis. The y-axis is obtained by the reciprocal of the uptake rate at that time (1/ v). These data were then plotted and a linear regression was fit. The Michaelis-Menten parameter of V_{max} is taken as the reciprocal of the y-intercept, K_m is obtained by multiplication of the slope by V_{max}.

When $C<<K_m$, C in the denominator of equation [3-1] can be ignored and equation [3-1] can be modified to

$$v = kC \qquad [3\text{-}4]$$

where $k = V_{max}/K_m$. These equations were used to describe v at any given concentration above ambient along the depletion curve. The first-order rate constant (k) describes a linear relationship for v vs. C at $C<<K_m$ and has units min^{-1}.

Overall P depletion during an experimental period was best described by first-order kinetics. The following integrated form of equation [3-4] was used to obtain P uptake coefficients

$$C = Co\, e^{-kt} \qquad [3\text{-}5]$$

where C is P concentration at any time, Co is the initial solution P concentration, and k is the first order rate constant.

Tracer Studies of Phosphorus Partitioning in Periphyton

Benthic periphyton and surface water collected in bulk from the field site on June 8, 1995, were used in the following laboratory studies to determine the partitioning of added ^{32}P into biotic and abiotic components of BP. These experiments were conducted on June 13 and 14, 1995.

Benthic periphyton of known fresh weight (0.5 g) was added to 50 ml (four replications per treatment) of 0.2 µm filtered water to maintain about a 1:100 fresh-weight BP to water ratio. Depending on the experiment, either ^{32}P as H_3PO_4 in 0.02 *M* HCl, or as 5'-[γ-^{32}P]ATP (DuPont NEN) were added. The inorganic ^{32}P was carrier-free. The stock [^{32}P]ATP had an activity of 111 TBq mM^{-1}. Additions of 0.2-0.7 kBq were made to each vessel. Unlabeled ATP or KH_2PO_4 was also added to manipulate the ^{31}P concentration of several treatments. The ^{31}P was either maintained at ambient ($P_i = 0.13$ µ*M*, $P_o = 0.32$ µ*M*) concentrations or was spiked to 3.2 µ*M* above ambient ($P_i = 3.4$ µ*M*, $P_o = 3.6$ µ*M*). One set of vessels were incubated in the light booth (10 W m^{-2}), while another set was incubated in the dark. The duration of the incubations was 1 and 12 h for the vessels which received inorganic P and 4 and 12 h for the organic P incubations.

Replicated distilled water blanks were also spiked with ^{32}P and were sampled periodically to provide control activities. After timed incubation, vessel contents were 0.2 µm filtered and 100 L of the filtrate was added to 10 ml of scintillation cocktail (Scinti-Verse I, Fisher Scientific). Radioactivity was determined with a Beckman liquid scintillation counter (LSC) (LS3801, Beckman Instruments Inc.). The BP and the membrane filter were placed into 50 ml of 0.01 *M* HCl. The BP was washed from the filter by gentle spraying of the dilute acid. The BP in dilute acid was stirred occasionally for the next 2 h. After this dilute acid extraction, the solution was again filtered (0.2 µm) and a 100 µl subsample was added to 10 ml scintillation cocktail and analyzed by LSC. The initial activity was partitioned into water activity, abiotic periphyton activity, and biotic periphyton activity. Activity remaining in solution after the uptake period was considered water activity. Initial activity minus water activity was the total activity in BP. Total BP activity was further separated into the activity extracted with 0.01 *M* HCl, the abiotic activity, and the biotic activity. Biotic activity was calculated by difference.

Results

Characterization of Periphyton

Total P contents of the periphyton types were in the order BP > MP > EP (Table 3-1). The chemical contents of each periphyton type remained essentially the same from time of collection until experimentation. This suggests that culturing under lights for 5 weeks did not appreciably change antecedent chemical conditions of the periphyton. The TP of the BP was similar for all dates except the June 13 - 14, 1995, laboratory studies where the TP was generally about 20% lower than that used at other times. Chemical composition of MP is approximately equidistant between that of EP and BP, suggesting the continuity of periphyton types. Ash contents are of the order EP > MP > BP indicating that EP is more encrusted with $CaCO_3$ than the other forms. Total C and TOC increased with decreasing ash content. Total N:TP ratio was between 69 - 106, with higher values for EP than for MP or BP. Total N increased proportionally to an increase in TOC content and was between 8 - 11% of TOC for all samples. Total P increased with increasing TC and TOC and inversely to TIC and ash content.

Extraction Procedure

Total dissolved P and DRP concentrations extracted from BP with 0.01 *M* HCl were not significantly different when statistically analyzed by a t-test ($p = 0.05$). The 0.01 *M* HCl extractant completely solubilized all added KH_2PO_4 as evidenced by >100%

Table 3-1. Selected chemical characteristics of periphyton used in the uptake studies.

Experiment Date, Periphyton Type	Total P	Total C	Total inorganic C	Total organic C	Total N	Ash Content
	mg kg^{-1}	g kg^{-1}				
Laboratory						
October 20, 1994						
EP	104	218	105	113	11	619
MP	189	247	104	143	13	541
BP	289	298	96	202	20	434
October 24-26, 1994						
EP	104	223	105	118	11	603
MP	214	270	101	169	16	506
BP	284	318	95	222	21	429
November 23-24, 1994						
EP	109	221	103	118	11	611
BP	273	295	95	200	20	445
Field						
June 7-8, 1995						
BP	272	282	96	187	23	472
^{32}P						
June 13-14, 1995						
BP (P_i)	233	276	96	180	23	471
BP (P_o)	222	268	96	173	21	473

recovery in DRP analysis of the $CaCO_3 + KH_2PO_4$ treatment and in accounting for 50 - 60% DRP recoveries when added to $CaCO_3$ and the organic P forms (Table 3-2). If all P_i were extracted without any hydrolysis of the organic forms, then expected recoveries during DRP analysis would be 50%. The low recoveries (<12%) of DRP in the $CaCO_3$ + P_o treatments suggests that organic P was not effectively hydrolyzed by the extractant.

The organic P forms were recovered after digestion and analyzed as TDP. The $CaCO_3$ control (no added P) shows that the $CaCO_3$ in the mixtures added small amounts of P to the extracted solutions. Total dissolved P in extracted solutions showed recoveries between 85 - 115%.

Visual inspection of BP tissue under SEM shows that filaments of the untreated periphyton are encrusted with $CaCO_3$ spicules (Fig. 3-2). These spicules were absent in periphyton tissue treated with 0.01 M HCl. It is hypothesized that this $CaCO_3$ abiotically binds the P removed during the dilute acid extraction. The filaments remained intact after acid extraction, suggesting that the method does not cause extreme cell damage and subsequent release of cellular constituents.

Phosphorus Uptake by Periphyton: Laboratory Conditions.

The first experiment compared P uptake by three types of periphyton in solution with Co = 4.7 - 6.0 μM P. Dissolved reactive P depletion from solution shows that P uptake by EP was more rapid than MP and BP (Fig. 3-3). Benthic periphyton had the slowest uptake. During the first 45 minutes of the incubation period, DRP in the solution containing EP returned to ambient levels (0.28 ± 0.02 μM P, mean ± SE, n = 3). During this time period, the DRP levels in solution containing MP were 1.5 ± 0.2 μM, and 2.5 ± 0.1 μM for BP containing solutions.

The initial uptake rates were calculated from the linear portion of the depletion curves. When using the solution depletion technique for a specific nutrient, the nutrient

Table 3-2. Phosphorus recovered after a 2 h extraction of reagent grade chemicals with 0.01 *M* HCl in a nominal 1:100 solid:solution ratio. Extracted solutions were analyzed colorimetrically for dissolved reactive P (DRP) and digested by persulfate oxidation and analyzed for total dissolved P (TDP).

Pure Compounds Extracted	Expected TDP if all added P released and hydrolyzed during extraction	Extracted P recovered during DRP analysis	Extracted P recovered during TDP analysis	Percent of Total added P recovered during DRP analysis	Percent of Total added P recovered during TDP analysis
	--------------	μ*M*	--------------	-------- %	--------
$CaCO_3$	0.0	0.05 ± 0.006	0.18 ± 0.010		
$CaCO_3$ + KH_2PO_4	0.92	1.05 ± 0.013	1.06 ± 0.074	114	115
$CaCO_3$ + Glucose 6-phosphate	1.85	0.07 ± 0.003	0.84 ± 0.090	8	91
$CaCO_3$ +KH_2PO_4 + Glucose 6-phosphate	1.85	1.02 ± 0.010	1.56 ± 0.061	55	85
$CaCO_3$ +Adenosine triphosphate	0.92	0.11 ± 0.013	0.87 ± 0.055	11	94
$CaCO_3$ +KH_2PO_4 + Adenosine triphosphate	0.92	1.08 ± 0.003	1.65 ± 0.094	59	89

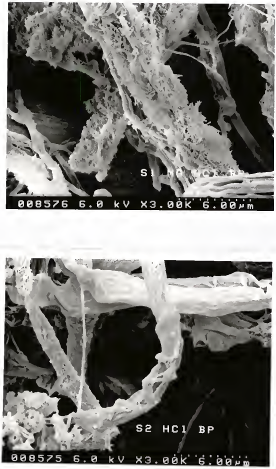

Fig. 3-2. Scanning electron micrograph of typical filament from benthic periphyton (BP) showing $CaCO_3$ encrustation (upper) and a typical filament in BP after 0.01 M HCl extraction (lower). Note lack of encrustation after 2 h treatment with dilute acid.

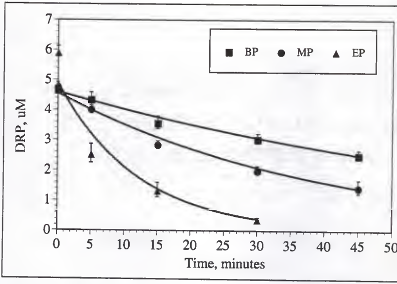

Fig. 3-3. Depletion of solution dissolved reactive P (DRP) concentration, normalized for solution volume and periphyton dry weight, during a laboratory experiment conducted October 20, 1994. Curves describing first-order kinetics were fit to the depletion data.Fig. 3-3.

should not become limiting (Reuter et al., 1986). With few exceptions, the solution P content in reaction vessels was not reduced by > 50% during any of the initial uptake rate experiments. Depletion during the linear phase was usually around 30% of the initial concentration.

Initial uptake rates for three periphyton types were; 0.127 ± 0.010 µmol DRP g^{-1} min^{-1} for EP, compared to 0.106 ± 0.042 µmol DRP g^{-1} min^{-1} for MP, and 0.072 ± 0.007 µmol DRP g^{-1} min^{-1} for BP. Dissolved reactive P depletion from solution was best described by first-order kinetics with rate constants in the order EP > MP > BP (Fig 3-3). Phosphorus uptake by EP did not fit first-order kinetics as well as did MP or BP. Uptake by EP can accurately be described by a 2-phase kinetic model with the initial uptake rate, given above, describing uptake in the first 5 minutes, and a slower uptake of approximately 0.077 µmol DRP g^{-1} min^{-1} for the next 25 minutes.

In the next experiment, uptake rates by three periphyton types were determined for one time step at 15 initial concentrations (Co = 0.2 - 62.2 *µM* P). Results showed a wide range in P uptake rates: EP = 0.039 - 0.623 µmol P g^{-1} min^{-1}, MP = 0.028 - 0.397 µmol P g^{-1} min^{-1} , and BP = 0.017 - 0.195 µmol P g^{-1} min^{-1} . At ambient levels (about 0.22 *µM* P) the change in solution concentration was minimal and was within the analytical variability for DRP. Thus, uptake was not determined at ambient concentration. For all periphyton types initial uptake rate was dependent on the initial P in solution. In all cases initial uptake was in the order EP > MP > BP, with EP generally being about 2-3 times higher than the other two types.

Initial uptake rates were used to generate Lineweaver-Burk plots to obtain an estimate of Michaelis-Menten uptake parameters. Maximum predicted uptake rate (V_{max}) and the Michaelis constants (K_m) were in the order EP > MP > BP (Table 3-3). Using resultant V_{max} and K_m values, initial uptake rate data were fit to predicted Michaelis-Menten plots (Fig. 3-4). There was generally good agreement between measured and predicted values. Phosphorus uptake by EP followed Michaelis-Menten

Table 3-3. Michaelis-Menton uptake parameters for P uptake by periphyton.

Experiment Date and Periphyton Type	Initial Concentration µM DRP† range	V_{max}	K_m	r^2
Laboratory Conditions		µmol g^{-1} min^{-1}	µ*M*	
October 24 -26, 1994.				
EP	0.3 - 62.2	0.852	9.9	0.96
MP	0.2 - 40.7	0.248	4.1	0.96
BP	0.2 - 38.2	0.101	2.5	0.87
November 23-24, 1994.				
EP	0.4 - 14.5	0.198	1.4	0.86
BP	0.6 - 7.7	0.038	1.1	0.94
In situ Conditions				
June 8, 1995.				
BP	1.5 -10.6	0.640	5.3	0.80
June 7. 1995. TDP†				
BP	5.5 - 10.1	0.661	21.4	0.99

† = µM total dissolved P (TDP) concentration for the June 7, 1995, P_o uptake study.

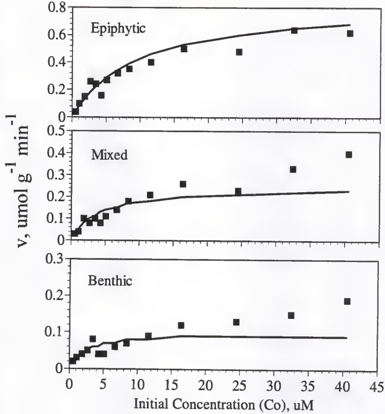

Fig. 3-4. Plots of uptake rate (v) versus initial dissolved reactive P (DRP) concentration. Based on initial uptake rates for laboratory experiments conducted October 24-26, 1994. Lines represent theoretical Michaelis-Menton values.

kinetics to a greater extent than either MP or BP. The V_{max} of the predicted Michaelis-Menten kinetics tends to underestimate that attained for MP and BP at solution P concentrations greater than about 15 μM.

A third experiment was conducted using the periphyton collected on October 18, 1994, and cultured for a period of five weeks at ambient DRP levels in a laboratory light booth (12 h light/dark cycle, light intensity = 10 W m^{-2}). At initial P concentrations (Co = 0.4 -14.5 μM), DRP levels in EP containing solution reached ambient levels (0.4 μM P) within 120 minutes (Fig. 3-5). Depletion of DRP followed an exponential decrease, being most rapid initially and slowing with time. Phosphorus depletion in BP containing solutions was slower than EP. Like EP, P depletion in BP containing solutions was rapid during early samplings (< 60 min), followed by exponential decrease with time. It took up to 240 minutes to reach ambient P levels at Co <6.7 μM. The treatment with the highest initial DRP concentration (7.7 μM) approached, but did not return to, ambient concentrations within 480 minutes. As with the previous experiments, these data also suggest that DRP uptake by EP to be greater than that of BP.

Time increments to determine initial uptake rates of the EP and BP curves ranged from 10 - 60 min, and from 10 - 120 min, respectively. There was generally a trend that uptake rate increases with initial concentration. Initial uptake rates for EP ranged from 0.051 - 0.176 μmol P g^{-1} min^{-1} over the initial concentration range of 1.1 - 14.5 μM. The ranges of BP uptake rates were between 0.012 - 0.036 μmol P g^{-1} min^{-1} . Again, the uptake rate increased slightly with increasing initial DRP concentration. The BP initial uptake rates increased from the Co = 0.6 - 2.5 μM, and then tended to maintain around 0.032 μmol P g^{-1} min^{-1} in the initial concentration range of 3.2- 7.7 μM. Michaelis-Menten parameters were obtained from the initial rate data. The resultant V_{max} and K_m are presented in Table 3-3.

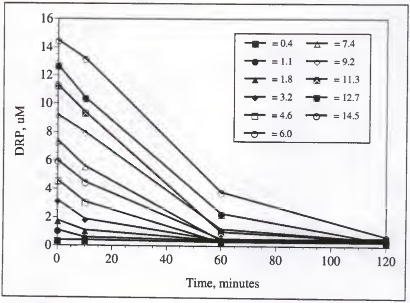

Fig. 3-5. Depletion of solution dissolved reactive P (DRP) concentration, normalized for solution volume and periphyton dry weight, during a laboratory experiment conducted November 23, 1994. Samples were collected at time increments from 0 to 480 minutes. Graph was truncated since all vessels returned to ambient concentrations (about 0.4 μ*M*) within 120 minutes.

The rapid depletion of solution DRP did not allow exponential curve fitting for the EP treatments. Depletion of solution DRP was described only by the initial uptake rates (Fig. 3-5). Curves resulting from the BP uptake experiment (Fig. 3-6) produced first-order rate constants ranging from 0.006 - 0.02 min^{-1} (Table 3-4). The rate constants were generally between 0.01 and 0.02 for initial concentrations Co = 0.60 - 4.0 μM. The rate constants were lower at Co > 4.0 μM, with a slight tendency for the rate constants to decrease with increasing initial concentration.

Phosphorus Uptake by Periphyton: Field Conditions

Inorganic P uptake

Rapid depletion of P was observed at Co > 1.5 μM. At ambient P levels (0.19 μM) no detectable change in concentration was observed. Ambient concentrations of about 0.19 μM were reached within 30 minutes when the initial solution P was 1.5 ± 0.1 and 2.7 ± 0.1 μM (Fig. 3-7) (mean ± SE, n = 4). After 60 minutes, solution P concentrations for treatments with Co = 5.6 ± 0.1 and 10.6 ± 0.2 μM were reduced to 0.7 ± 0.1 and 1.7 ± 0.1 μM respectively.

Initial uptake rates were determined by depletion of solution DRP in 5 min. Initial uptake rates ranged from 0.077 ± 0.007 to 0.429 ± 0.036 μmol P g^{-1} min^{-1}. There was a distinct trend of increasing uptake rate with increasing concentration. Michaelis-Menten parameters show that the predicted K_m value is several times higher than the ambient P level (Table 3-3).

First-order rate constants were obtained from the depletion curves for treatments with Co > 1.5 μM (Fig. 3-7 and Table 3-4). The first-order rate constants were in the range of 0.03 -0.07 min^{-1}, and showed a decreasing trend with an increase in DRP concentrations.

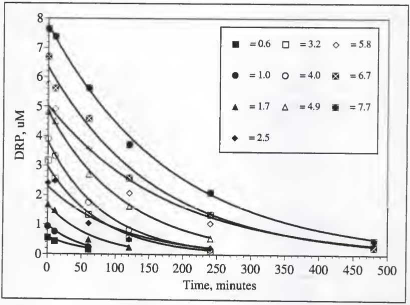

Fig. 3-6. Depletion of solution dissolved reactive P (DRP) concentration, normalized for solution volume and periphyton dry weight, during a laboratory experiment conducted November 24, 1994. Curves describing first-order kinetics were fit to the depletion data.

Table 3-4. Predicted initial P concentration, rate constant, and r^2 for first-order equations of the form $C= Co\ e^{-kt}$ fit to depletion curves.

Experiment Date, Periphyton Type, and Initial Concentration µ*M* DRP† (mean ± SE)	Predicted Initial Concentration (Co)	First-order Rate Constant (k)	r^2
Laboratory Conditions	µ*M*	min^{-1}	
October 20, 1994.			
EP 6.0 ± 0.2	4.9	0.0841	0.98
MP 4.8 ± 0.1	4.6	0.0261	0.99
BP 4.7 ± 0.2	4.6	0.0135	0.99
November 24, 1994. BP			
0.6	0.6	0.0172	1.00
1.0	1.0	0.0203	1.00
1.7	1.7	0.0153	0.97
2.5	2.3	0.0096	0.96
3.2	3.0	0.0124	1.00
4.0	3.8	0.0120	1.00
4.9	4.8	0.0088	1.00
5.8	5.0	0.0057	0.98
6.7	6.3	0.0064	1.00
7.7	7.8	0.0056	1.00
In situ Conditions			
June 8, 1995. BP			
1.5 ± 0.1	1.4	0.0529	0.94
2.7 ± 0.1	2.8	0.0708	1.00
5.6 ± 0.1	5.7	0.0323	0.97
10.6 ± 0.2	9.9	0.0302	0.99
June 7. 1995. BP, TDP			
3.3 ± 0.2	ND		
5.5 ± 0.1	5.5	0.0318	1.00
10.1 ± 0.2	10.9	0.0199	0.98

† = µ*M* total dissolved P (TDP) concentration for the June 7, 1995 P_o uptake study.

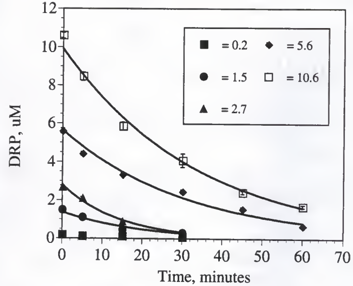

Fig. 3-7. Depletion of solution dissolved reactive P (DRP) concentration, normalized for solution volume and dry weight benthic periphyton (BP), for field experiment conducted June 8, 1995. Curves describing first-order kinetics were fit to the depletion data.

Organic P uptake

The uptake of P_o by BP was studied in situ on June 7, 1995. Solutions were spiked with ATP at four initial concentrations (0.9 - 10.1 μM). Changes in P concentrations of the water show that virtually all of the added ATP was hydrolyzed to DRP within 15 min (Fig. 3-8). This occurred in all BP treatments and in a water-only control (Co = 3.8 μM). Once hydrolyzed the P concentration in the BP-containing treatments decreased rapidly, but the P in the control treatment remained constant. Generally, P concentrations returned to ambient levels within 120 minutes for all BP-containing treatments (Fig. 3-8).Initial uptake rates were determined at the 15 min time step for all concentrations > ambient (0.9 μM). One replicate in the Co = 10.1 μM treatment was omitted as an outlier, therefore n = 3, all other treatments n = 4. The initial uptake rates varied between 0.099 ± 0.024 μmol TDP g^{-1} min^{-1} for Co = 3.3 μM to 0.203 ± 0.016 μmol TDP g^{-1} min^{-1} for Co = 10.1 μM. Initial uptake rate increased with increasing concentration. Michaelis-Menten uptake parameters were derived from these results (Table 3-3). Exponential equations, fit to points with P concentration > than ambient for the treatments with Co = 5.5 and 10.1 μM, produced first-order rate constants of 0.03 - and 0.02 min^{-1} respectively (Table 3-4).

Phosphorus Partitioning in Periphyton

Benthic periphyton, brought from the field site on June 8, 1995, was subjected to laboratory ^{32}P uptake studies. A higher proportion of available P was taken into the abiotic fraction at ambient P concentrations, $P_i = 0.13\ \mu M$ and $P_o = 0.32\ \mu M$, than at spiked concentrations, $P_i = 3.4\ \mu M$, $P_o = 3.7\ \mu M$. This result occurred irrespective of irradiance, P-form, or incubation time (Fig. 3-9 and 3-10). At ambient concentrations, >20% of $^{32}P_i$ activity was in the abiotic compartment after 1 h, compared to <6.5% at spiked concentrations. In 12 h the ambient abiotic P pool had >8% $^{32}P_i$ activity; whereas, at Co = 3.4 μM <3% $^{32}P_i$ activity was in this fraction (Fig. 3-9). Similarly, at

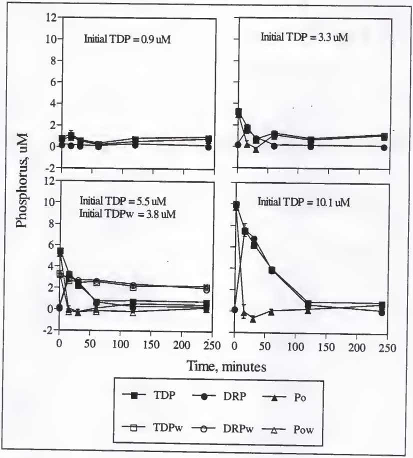

Fig. 3-8. Concentrations of total dissolved P (TDP), dissolved reactive P (DRP), and organic P (P_o) (by difference), normalized for volume and dry weight benthic periphyton (BP) during field Po uptake experiment. Unfiltered WCA-2A-217 water was spiked to 0.9 (ambient), 3.3, 5.5, and 10.1 μM TP with ATP. Unfiltered water controls (UFW) devoid of BP were also run at Co = 3.8 μM (TDPw, DRPw, Pow). This study was conducted on June 7, 1995. Points indicate mean solution concentration (±SE, n=4).

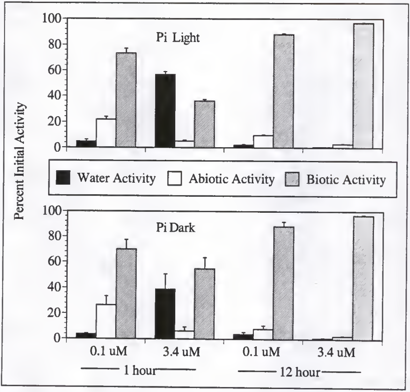

Fig. 3-9. Partitioning of added ^{32}P between solution and periphyton. Benthic periphyton (BP) was incubated in 0.2 µm filtered WCA-2A-217 water to which ^{32}P was added as carrier-free H_3PO_4 for either 1 or 12 h. Spiked treatments involved additions of 3.23 μM ^{31}P as KH_2PO_4 to bring Co = 3.4 µM. Concurrent sample sets were incubated under light and dark conditions. After the uptake experiments the BP was collected by filtration and extracted with 0.01 M HCl for 2 h. The activity released during extraction is considered that abiotically complexed. The activity not extracted (by difference) represents biotic incorporation. Bars represent mean ± SE, n = 4.

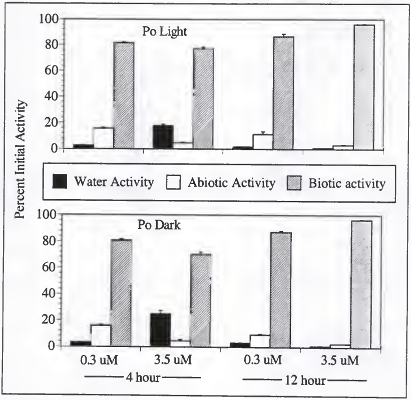

Fig. 3-10. Partitioning of added ^{32}P between solution and periphyton. Benthic periphyton (BP) was incubated in 0.2 µm filtered WCA-2A-217 water to which ^{32}P was added as adenosine 5'-(γ-^{32}P) triphosphate (3000 Ci mM^{-1}) for either 4 or 12 h. Spiked treatments also had additions of 3.23 µM ^{31}P as ATP to bring Co = 3.4 µM. Concurrent sample sets were incubated under light and dark conditions. After the uptake experiments the BP was collected by filtration and extracted with 0.01 M HCl for 2 h. The activity released during extraction is considered that abiotically complexed. The activity not extracted (by difference) represents biotic incorporation. Bars represent mean ± SE, n = 4.

ambient P_o concentrations, approximately 16% of the $^{32}P_o$ activity was in the abiotic pool after 4 h compared to 5% at spiked concentrations. By 12 h, 9-12% of the added activity was in the abiotic pool at Co = 0.3 μM TDP where about 3% was in this portion for Co = 3.5 μM TDP (Fig. 3-10). There was a shift in activity from the water and abiotic fractions to the biotic fraction with time. At 1 h incubation around 70% of total added $^{32}P_i$ activity for ambient P_i treatments and 36-55% of the activity at Co = 3.4 μM DRP was in the biotic compartment. By 12 h, over 88% of total added activity was in the biotic portion for all P_i treatments. The $^{32}P_o$ fraction contained in the biotic compartment was 70 - 82 % of the total activity at 4 h incubation and increased to 86 - 97 % by 12 h (Fig. 3-10). Irradiance had little effect on the partitioning of ^{32}P activity in either P_i or P_o treatments.

Discussion

Characterization of Periphyton

Total P content of periphyton used in uptake experiments ranged 104 mg kg^{-1} to 289 mg kg^{-1} (dry wt). Other researchers have reported total P content in periphyton in the range of 100 - 400 mg kg^{-1} (Swift and Nicholas, 1987; Vymazal et al., 1994). The slough site sampled by Vymazal et al. (1994) was located closer to a gated culvert than was the WCA-2A-217 site from which the periphyton in the present experiments was obtained. Canals have been shown to effect the nutrient concentration of the periphyton, water, and soil such that concentrations generally decreased with increasing distance from the inflow (SWIM, 1992; Swift and Nicholas, 1987; Koch and Reddy, 1992; DeBusk et al., 1994). In fact, Vymazal et al. (1994) found TP contents in EP from a mixed sawgrass/cattail community near a culvert of WCA-2B averaged 671± 21 mg kg^{-1}.

Vymazal et al.(1994) suggested the degree of calcification inversely affected the P content of periphyton. In this study EP had the highest ash content, indicating the greatest degree of calcification (Table 3-1). Ash content can generally be used as an estimation of $CaCO_3$ content (Browder et al., 1994). Epiphytic periphyton total P contents were about 40% that of BP but, when normalized for differences in organic matter content (TOC), the TP content was about 70% that of BP.

The differences in P content of two periphytic forms may be due to the sources of P available to them. Benthic periphyton has been shown to utilize P from both the water column and the soil porewater (Hansson, 1989, 1992). The soil porewater at WCA-2A-217 has been shown to have higher equilibrium DRP concentrations than the overlying water (Koch and Reddy, 1992). Hansson (1992) observed that as the floodwater P concentration increases, the sediment porewater P utilization decreases. Given very low ambient floodwater DRP of the interior marsh ($< 0.16\ \mu M$) it seems plausible that any P source would be in demand. Epiphytic periphyton can receive P from the water column and the exudation of P from the macrophytic tissue. The contribution of P from healthy macrophytes was shown to be only 3 - 9% of the P in EP (Carignan and Kalff, 1982). Epiphytic periphyton in the Everglades has been shown to preferentially colonize dead macrophyte stems (Browder, 1981). Old or dead macrophyte tissues might release more P than healthy ones, therefore adding available P to EP; however, Riber et al. (1983) found that on a dry weight basis the EP grown on new *Phragmites spp.* stems had a higher P content than that on older stems.

The TN content of periphyton was between 11 and 23 mg TN g^{-1} (dry wt.). Like P, TN content increased in the order BP > MP > EP. The TN content of BP was consistent for all times. Lower TN values were reported for periphyton located in slough sites (Vymazal et al., 1994). In another study, the TN content of the periphyton from WCA-2A-217 was reported to be in the range of 9 - 36 mg g^{-1} (Swift and Nicholas 1987). The N:P ratio from 69 to 106:1, with the ratio generally higher in EP than in BP. Earlier studies have reported a wide range in N/P ratios (53 - 205) of periphyton sampled in WCA-2A (Swift and Nicholas, 1987; Vymazal et al., 1994). The high N/P ratio suggests that periphyton is P limited.

Ash content ranged 429 - 619 g kg^{-1} (dry wt.) with highest values for EP followed by MP > BP. The ash content in periphyton samples from the southern Everglades represented 49-81%.of the total mass and varied little between seasons (Browder et al., 1982). Vymazal et al. (1994) reported slough EP organic contents (as AFDM) as 318 - 525 g kg^{-1} which corresponds to an ash content of 48 - 68%. Müller (1983) reported ash content of 33-73% for a *Clodaphora* community obtained from sandstone substrata (epilithic) in a eutrophic lake in Sweden, with lowest values observed during a July sampling event when the total Ca content was at a maximum. In our study, the ash content was only slightly higher for BP during the summer sampling (June, 1995) than for a fall sampling (October, 1994).

Inorganic P Uptake

Several studies have identified the effect of P loading on species compositional changes (Swift and Nicholas, 1987; Radar and Richardson, 1992; Gleason and Spackman, 1974), on periphytic biomass production (Bothwell, 1989; Hansson, 1992;), transfers between substrate and periphyton (Carignan and Kalff, 1982; Riber et al., 1983; Hansson, 1989), and on P contents as affected by physical conditions (e.g. water velocity)(Horner et al., 1983; Loch and John, 1979). A few studies have described P uptake by periphyton on an area basis (Adey et al., 1993; House et al., 1995; Vymazal, 1988). Reuter et al. (1986) determined the kinetics of N uptake by epilithic periphyton. However, to my knowledge there are no reported studies on P uptake kinetics of periphyton.

The uptake estimates of K_m and V_{max}, were obtained by using the P depletion technique (Reuter et al., 1986). An advantage of this method is that measurements over a full range of external concentrations can be made with the same sample (Drew et al., 1984). The value of K_m represents the affinity an organism or a community has for a given substrate (Reuter, 1986). The lower the K_m, i.e. the lower the P concentration resulting in half the maximum uptake rate, the higher is the affinity of P. Uptake rates are sensitive at concentrations lower than K_m (Epstein, 1972). These values have been used in algal competition studies as a measure of a species advantage for limited P (Grover, 1989; Bentzen and Taylor, 1991a). The maximum uptake velocity (V_{max}) is a capacity factor denoting the maximum uptake rate when all carrier sites are loaded (Epstein, 1972). A high capacity for nutrients, i.e. a large V_{max}, is considered advantageous in nutrient rich environments in that it allows maximal exploitation of the available substrate (Cotner and Wetzel, 1991).

The half-saturation constants (K_m) predicted for P_i uptake varied between 1.1 μM and 9.9 μM. The K_m values obtained during the 1994 experiments suggest that BP has a higher affinity for low DRP concentrations than does EP. A few studies have sought to determine the uptake kinetics of filamentous algae. Borchardt et al (1994) reported K_m values for *Spirogyra fluviatilis* Hilse to be 0.4 - 1.5 μM P, slightly lower but of a similar magnitude to those reported here. Tarutani and Yamamoto (1994) reported Km = 0.7 μM for P_i uptake by the marine diatom *Skeletonema costatum*. Using whole water samples from a Canadian shield lake, Lean and White (1983) obtained K_m values for phytoplankton ranging 2.4 - 9.5 μM P when K_m was calculated from standard ^{31}P additions of up to 20 $\mu g\ L^{-1}$ and 0.3 - 4.0 μM when calculated from ^{32}P additions. Cotner and Wetzel (1992) reported much lower K_m values for whole water phytoplankton P_i uptake studies of between 0.05 to 0.19 μM.

The K_m values obtained for periphyton are high, being 1 to 2 orders of magnitude greater than the ambient DRP concentration typical of this wetland (< 0.16 μM). Reuter et al. (1986) found K_m values for nitrate and ammonium uptake by periphyton in Lake Tahoe to also be 1-2 orders of magnitude greater than typical ambient concentrations. Their results seemed to contradict those found for phytoplankton in the same setting causing them to conclude that care must be taken when generalizing between phytoplankton and periphyton communities, even in the same water body.

The calculated maximum uptake rates (V_{max}) ranged 0.04 - 0.85 µmol g^{-1} min^{-1}. The highest reported value was for EP. The in situ V_{max} for BP (June, 1995) approaches this maximum. These data suggest a higher capacity of EP to take up P when P is present in excessive concentrations and that V_{max} is dependent on the physiological state of the periphyton. However, under ambient condition, P uptake rate will not approach V_{max}, as normal ambient DRP and TP concentrations are around 4 and 10 µg L^{-1}, respectively (Swift and Nicholas, 1987).

The estimate of V_{max} predicted by fitting initial uptake rates at concentrations from ambient to about 40 µ*M* DRP (Fig. 3-4) show that at higher initial concentrations (> ca. 10 µ*M*) the predicted V_{max} underestimates the experimentally determined uptake rate. The reasons for which two seemingly different uptake rates would result as initial P concentration increases is unknown. An analogy between P uptake by MP and BP and that of P adsorption to $CaCO_3$ may lead to speculation of possible mechanisms involved. It has been reported that the retention of P to $CaCO_3$ occurs initially by rapid filling of monolayer sorption sites followed by slower rearrangement of phosphate ions in $CaCO_3$ crystal growth (Berkheiser et al., 1980; Griffin and Jurniak, 1974). As the initial solution P increases, precipitation of $CaHPO_4$ may be favored (Berkheiser et al., 1980). A possible mechanism to explain the V_{max} predictions (Fig. 3-4) is that biotic uptake and P adsorption to $CaCO_3$ occurs at all concentrations; however, as the initial P concentration increases Ca-P precipitation reactions have a greater affect on P removal from solution.

Turnover times, calculated as the reciprocal of the first-order constant (k), ranged 14 - 180 min (Table 3-4). At DRP concentrations ca. 4.8 µ*M*, turnover time was 12 min for EP and 38 min for MP. The turnover time of BP during this experiment was 74 min. Turnover times increased with increasing concentration. Lean and White (1983) found a P turnover time in whole water phytoplankton studies to be 5.5 min at ambient P levels

(<1 μg L^{-1}), but that the turnover time increased to 20 min with the addition of only 2 μg P L^{-1}. They suggested that at very low P concentrations rapid uptake and exchange of P was primarily due to bacteria and microalgae; at higher concentrations uptake is by larger algae or clumps of algal cells (Lean and White, 1983)

The rates of P_i uptake varied between the October and November 1994 experiments. Initial uptake rates compared for a given periphyton type at a similar initial concentrations were generally in close agreement for the October 20, and October 24 -26, 1994 experiments (data not shown). However, incubation under artificial light (10 W m^{-2}) for a period of about 5 weeks affected the uptake ability of EP and BP. For both periphyton types, uptake rates were about one-half of those measured for the October experiments. The October uptake rates for BP ranged 0.033 - 0.084 μmol P g^{-1} min^{-1}, while those of the November experiment were within the range of 0.018 - 0.036 μmol P g^{-1} min^{-1}. The uptake rates for BP at similar concentrations measured in the field during June 1995 were 0.077 - 0.429 μmol P g^{-1} min^{-1}. Similarly, the EP uptake rates at the October sampling with initial concentrations of 4.6 - 12.6 *μM* were 0.162 - 0.348 μmol g^{-1} min^{-1}, and those of November were 0.092 - 0.173 μmol g^{-1} min^{-1}. The ability of periphyton to maintain the uptake rates of the October experiments was reduced during laboratory incubation. There is not a corresponding change in the chemical characteristics studied (Table 3-1). For example, if the degree of calcification reduces the ability of periphyton to obtain P, then a reduced ash content in the November periphyton could explain the reduced uptake. Ash content, and presumably the degree of calcification, were maintained during this period.

The mass of DRP removed by BP during the field study of June 1995 was greater than that of either the October or November 1994 laboratory BP uptake studies. These results suggest that seasonal variation in BP uptake ability, or in variations due to experimental conditions (e.g. field vs. laboratory), or a combination of these factors, influence P uptake rates.

The material used in laboratory experiments was collected on October 18, 1994, when the water level at WCA-2A-217 was uncharacteristically high (about 1 meter). The BP collected at this time showed visual symptoms of stress. The EP, being under relatively less water depth due to its position in the water column showed no apparent stress. Gleason and Spackman (1974) and Browder et al. (1994) suggested optimal conditions for benthic calcareous periphyton mats in the Everglades develops in water depths < 0.6 m. In deeper water, or where shading is excessive, the mats will degenerate to "a crumbly mass or a thin coating of algae" and will begin to take on a deeper green appearance (Gleason and Spackman, 1974). The water depth at WCA-2A-217 on June 7, 1995 was approximately 0.4 m. The benthic periphyton at this time had the normal golden appearance. The bulk chemical parameters measured for periphyton used (Table 3-1) did not change appreciably with time for each type, but it is likely that the proportion of biologically active portion is affected by storage time and seasonality.

Organic P Uptake

Phosphorus as ATP was used to determine the uptake rate of P_o by BP under in situ conditions in June 1995. Bentzen and Taylor (1991b) found [^{32}P]ATP (as a model phosphomonoester, PME) was an effective substrate for tracing P_o dynamics in phytoplankton in a manner comparable to using $^{32}PO_4^{3-}$ to study PO_4^{3-} dynamics.

Added ATP had been rapidly hydrolyzed to DRP within 15 min. Since all ATP was hydrolyzed by the first sampling, the actual hydrolysis rate could not be determined. However, based on the maximum ATP addition of ca 7.0 μM P (217 μg L^{-1}) and a 15 min time period, the calculated hydrolysis rates were > 0.47 μM (14.5 μg L^{-1} min^{-1}). The in situ uptake studies showed similar maximum uptake rates for P_i and P_o (P_i V_{max} = 0.64 μ mol g^{-1} min^{-1}, and 0.66 μmol g^{-1} min^{-1} for P_o). Bentzen et al. (1992), found that PME (ATP) and PO_4^{3-} uptake by phytoplankton to be comparable, with PO_4^{3-} uptake being slightly greater. The positive, significant relationship between these substrates led them to conclude that similar forces are involved in P_i and P_o utilization (Bentzen et al., 1992)

As is the case with P_i uptake, there is virtually no research to date on the uptake and utilization rates of P_o by periphyton. Numerous studies have been conducted on the uptake of P_o by plankton and, discussion of seemingly analogous mechanisms may explain P_o utilization by periphyton. Several studies have shown the utilization of P_o by algal and bacterial components of plankton (Bentzen et al., 1992; Bentzen and Taylor, 1991b; Cotner and Wetzel, 1992; and Ammerman and Azam, 1985). Naturally occurring P_o, in the form of phosphomonoesters (PME) is not readily available for algal or bacterial (and by analogy periphyton) uptake until it is converted to inorganic P. Phosphomonoesters are hydrolyzed enzymatically to P_i by several phomonoesterases. The most widely recognized phosmonoesterase in aquatic systems is the non-specific alkaline phosphatase (AP) (Bentzen et al., 1992). However, Ammerman and Azam (1985) reported that marine and freshwater bacterial plankton have a cell surface 5' nucleotidase that rapidly hydrolyzes 5' nucleotides and regenerates P_i. They suggest that this enzyme activity might also be a result of algae and cyanobacteria, but that 5' nucleotidase has not yet been reported in photoautotrophs (Ammerman and Azam, 1985). A number of factors influence the hydrolysis of PME including; the amount and availability of P containing organic compounds, the overall P demand, the rate of phosphatase enzyme production, and the amount and availability of P_i (Syers et al., 1973; Ammerman and Azam, 1985; Cotner and Wetzel, 1991). Uptake of P from PME can enter a cell directly if the ecto-phosphomonoesterase is closely associated to the P transport mechanism (Lugtenberg, 1987 as cited in Bentzen et al. 1992) or indirectly if hydrolyzed PO_4^{3-} is released to solution or if some enzyme is free in solution (Bentzen et al., 1992). Once hydrolyzed, the P_i is then available for uptake either by the organism that hydrolyzed it or by other organisms. For instance, in California coastal water 85 - 90 % of the P_i hydrolyzed by bacterial 5' nucleotidase was released to the environment where it could supply an estimated 50 - 100 % of the P_i consumption by phytoplankton (Ammerman and Azam, 1985). Cotner and Wetzel (1991) isolated bacterial fractions producing AP activity along a transect from the littoral to the pelagic zone of a lake they studied and found the highest AP activity was in the bacterial isolate from the littoral zone. WCA-2A-217 shares more similarities to a lake littoral zone than to the pelagic waters. Inorganic P_i as PO_4^{3-} has been shown to inhibit the activity of phosphomonoesterases such as AP (Bentzen et al., 1992; Cotner and Wetzel, 1991); however, Ammerman and Azam (1985) found 5' nucleotidase to be relatively insensitive to high concentrations of P_i. They observed no inhibition at P_i concentrations below 100 μM (Ammerman and Azam, 1985). The P_i concentrations at WCA-2A-217 never approaches 100 μM; therefore, if this mechanism exists at WCA-2A-217, then the enzyme should be active at all times. Benzten et al. (1992) and Cotner and Wetzel (1991) also suggested that even with P_i inhibition, P_o hydrolysis occurs when the P demand is high.

Orthophosphate is considered to be the P form preferentially utilized in aquatic systems; however, the available fraction of P_o, as exemplified by ATP, is also actively utilized by Everglades BP. The rapid hydrolysis rates, the rapid uptake rates, and a maximum uptake rate similar to that of P_i all indicate that P from PME is in high demand at WCA-2A-217.

Partitioning of ^{32}Phosphorus

Rates measured by solution depletion estimate P uptake by periphyton without differentiating the mechanisms involved. We hypothesized that an abiotic interaction, mediated by periphytic activity occurs between Ca^{2+}, $CaCO_3$, and PO_4^{3-}, and that this interaction is at least partially responsible for removing P from solution. To test this hypothesis, BP was incubated under laboratory conditions in solutions containing either $^{32}P_i$ as H_3PO_4 or [^{32}P]ATP.

In short-term incubations (1 and 4 h for P_i and P_o, respectively) and at ambient concentrations, greater than 70% ^{32}P activity was biotically incorporated (Fig. 3-9 and 3-10). Ratios of biotically incorporated ^{32}P activity were similar between light and dark incubations.

The activity associated with the abiotic compartment was greater for $^{32}P_i$ than for [^{32}P]ATP during the short-term incubations. This may be due to a shift from abiotic compartment to biotic with time (4 h vs. 1 h) or may suggest a higher affinity for P_o uptake by biotic mechanisms. Similarly, abiotic mechanisms may be better able to complex P_i than P_o. As mentioned, P_o is not readily taken up until it is hydrolyzed to P_i by ecto-phosphatases. During this hydrolysis the majority of the [^{32}P]ATP is associated with cell surfaces, and if the ecto-phosphatases are close to the transport system, the [^{32}P]ATP would be incorporated into the biotic compartment before it has an opportunity to associate with $CaCO_3$. That [^{32}P]ATP which appeared in the abiotic compartment was possibly hydrolyzed by free enzymes in solution.

Periphyton at WCA-2A-217 is dominated by filamentous cyanobacteria and hardwater diatoms (Swift and Nicholas, 1987). Everglades cyanobacteria have the ability to precipitate $CaCO_3$ (Gleason, 1972; Gleason and Spackman, 1974; Browder et al., 1994). Several researchers have shown P to coprecipitate with $CaCO_3$ in freshwaters during periods of high algal activity, or in water that mimics such activity (House et al., 1995; Otsuki and Wetzel, 1972; Murphy et al., 1983). Diaz et al. (1994) observed rapid precipitation of P as Ca-P in Everglades water when pH was manipulated to > 8.0. The conditions that favor periphytic $CaCO_3$ encrustation and subsequent P coprecipitation are high calcium concentrations, high alkalinity, high pH, low partial pressure of CO_2, high levels of $CaCO_3$ saturation, and high temperatures (Gleason and Spackman, 1974; Browder et al., 1994; Otsuki and Wetzel, 1972; and House, 1990). Photosynthesis creates microenvironments around cells with conditions that favor rapid calcite formation (McConnaughey, 1991; Gleason and Spackman, 1974). Additionally, the algal surface acts to catalyze the calcite precipitation by providing the initial nuclei for further crystal growth (Müller, 1983; Murphy et al., 1983; Gleason and Spackman, 1974). The concentration of available P is also important but mostly from its inhibitory effect on calcite crystal growth. Depending on the level of supersaturation, solution P may stop crystal growth completely (House, 1987, as cited in House 1990). The $CaCO_3$-P coprecipitation reaction occurs when solution P becomes adsorbed onto $CaCO_3$ and becomes incorporated as the crystal grows (Griffin and Jurniak, 1974; House, 1990). This process is more important for removing P rather than P becoming adsorbed on the particulate $CaCO_3$ after the formation of crystals (Otsuki and Wetzel, 1972)

Other researchers have stated that $CaCO_3$-P coprecipitation is not responsible for the removal of P. Based on the inverse relationship between Ca and P contents Vymazal et al. (1994) promoted that P is bound to organic matter in periphyton rather than to calcite precipitations. They suggested that the relationships between algal calcite precipitation, P uptake, and calcium phosphate precipitation are unknown, but suggests $Ca_3(PO_4)_2$ precipitation is not directly linked to algal calcite precipitation. They further stated that calcareous periphyton does not function as a P storage mechanism as has been suggested by Gleason (1974). They allowed the possibility of water column calcium P precipitation, but that the direct $Ca_3(PO_4)_2$ precipitation by periphyton is probably not the mechanism. Recent analysis by X-ray diffraction of dried, ground BP, containing around 550 mg $CaCO_3$ g^{-1}, produced results very similar to that of pure $CaCO_3$ with no mineral forms (e.g. $Ca_3(PO_4)_2$) of calcium phosphates present (see Fig. 5-6). This does not exclude the possibility that P coprecipitates with $CaCO_3$ as an amorphous form, or that P precipitated in a mineral form with Ca occurs in concentrations too low to appear in X-ray diffraction. The inverse relationship between Ca and P contents can be explained by the differences in the density of highly calcareous periphyton to that of less calcareous periphyton. On a dry weight basis, the increased mass due to $CaCO_3$ is going to dilute the mass of all other elements.

The stability of P coprecipitated with $CaCO_3$ needs further study. Gleason (1972), Gleason and Spackman (1974) and others described the diurnal fluctuations in physicochemical parameters leading to $CaCO_3$ precipitation in the water column over periphyton communities. Generally, supersaturation and subsequent precipitation during the day was at least partially balanced by release during night-time undersaturation (Gleason and Spackman, 1974). Other factors, such as water depth (Gleason and Spackman, 1974) and increased levels of P have been shown to reduce the levels of calcification in periphyton. Vymazal et al. (1994) cited enrichment studies where the addition of P caused a disintegration of the calcareous mat but the dominant cyanobacteria remained. Several other researchers have shown a loss of the calcareous periphyton and a replacement by other algae species, mostly green algae after prolonged exposure to increased P nutrition (Swift and Nicholas, 1987; Steward, 1973; and Ornes and Steward, 1973 as cited in Vymazal et al., 1994). The fate of the P within the periphytic matrix is unknown.

I propose that P uptake by periphyton involves a number of simultaneous reactions. Phosphorus can move directly to cell surfaces where it is taken up biotically or, in the case of P_o, is first hydrolyzed and then taken up. Inorganic P is rapidly, but weakly complexed to the surface of $CaCO_3$ crystals. Much of this P eventually moves into the biotic compartment within a few hours. Some surface adsorbed P may become incorporated into more stable Ca-P if the adsorption occurs during active $CaCO_3$ crystal growth.

Conclusions

The evidence presented here indicates that the periphyton of the interior of WCA-2A-217 exists in a condition of P limitation. Parameters describing the P uptake by periphyton were obtained by the solution depletion technique for the linear portion of the depletion curves. Rate processes that are a function of substrate concentration, as were ours, are never truly linear, yet appear that way when uptake is relatively slow. Results showed no change in P concentration in ambient treatments; therefore, P uptake was only determined after enrichment. At ambient concentrations, the periphyton is in steady-state with the environment; and therefore, net community influx equals efflux. It is difficult to chemically determine differences in influx/efflux at ambient levels without the use of radioisotopes. The uptake rates after enrichment should not be interpreted as a measure of long-term uptake potential, but are useful in quantifying uptake rates for intermittent pulses of P enriched water and to determine the maximum P uptake rates (V_{max}) and half-saturation constants (K_m). Ambient DRP levels in the interior of WCA-2A are always below $K_{m;}$ and therefore, uptake rates are always $< V_{max}$. The periphyton of the interior of WCA-2A was shown to have rapid uptake rates and high capacities to assimilate P.

The $CaCO_3$ - P coprecipitation mechanism as represented by abiotic uptake did not remove a substantial percentage of added P during the short-term enrichment experiments. The precipitation of $CaCO_3$ by periphyton and the interaction of $CaCO_3$ with P could add P retention capacity to peat soils of WCA-2A-217, leading to long-term storage. The P within the periphyton will cycle internally. The abiotic P is also available to biotic consumption. Although the $CaCO_3$ - P coprecipitation did not prove to be an appreciable P removal mechanism the fact that this mechanism exists deserves further attention in the hopes of developing processes that increase P retention. Mat forming calcareous cyanobacteria are encouraged by short hydroperiods, shallow water depth, and increased levels of $CaCO_3$ saturation. Geochemical modeling of the conditions which may lead to enhanced calcification would aid in the evaluation of the $CaCO_3$ - P interaction and help to determine the long-term stability of this material.

CHAPTER 4
PHOSPHORUS FLUX BETWEEN SOIL AND WATER COLUMN

Introduction

The photosynthetic activity of periphyton can exert a strong influence on the physicochemical properties of shallow waterbodies, which in turn, can influence P exchange between the soil and the water column (Carlton and Wetzel, 1988). For instance, even at low light intensities (<50 µmol quanta m^{-2} s^{-1}), periphytic photosynthesis can produce marked changes in pH and oxygen content of surficial sediments (Revsbech et al., 1983; Carlton and Wetzel, 1988) which may regulate P flux to the water column (Boström et al., 1982). Carlton and Wetzel (1988) observed diurnal variations in P-flux from the sediment and believe these diel fluctuations tend to conserve P in the sediment.

In wetlands, periphyton can be present floating in the water column, growing on the surfaces of submerged macrophytes, or as a layer at soil/the floodwater interface. The latter type is known as benthic periphyton (BP). In the Everglades, the BP exists as a layer covering the soil surface. As such, the periphyton layer itself is often stratified; that is, conditions at the floodwater/ periphyton interface may not be similar to those at the periphyton/soil boundary. Sand-Jensen (1983) noted that the effects of external parameters (i.e., nutrient concentration) vary with the degree and thickness of mat development. He found that there is greater exchange with the external water environment when the periphyton layers were thin but as the BP layer thickens the magnitude of internal processes become more important.

The BP has the capacity to assimilate dissolved reactive P (DRP), thus regulating DRP concentration in the water column. Regulation of water column P is further affected by the periphyton mediated physicochemical properties. The calcareous BP is eliminated from Everglades wetlands under increased P loading (Swift and Nicholas, 1987). Elimination of this delicate biota removes the buffer between the soil porewater and the water column.

I hypothesize that BP will act as a barrier for soil pore water DRP thus reducing the flux of P into the water column. Elimination of this layer will increase P-flux from the soil to the overlying water column. The objectives of this study were to determine the influence of BP on: selected physicochemical properties of the water column, P-flux under ambient and P enriched water column conditions, and the fate of water column P under light and dark conditions. These objectives were studied via a series of field and greenhouse experiments using microcosms, soil-BP-water columns, and field enclosures.

Materials and Methods

Site Description

Field experiments and intact core collection were conducted at a site designated as 217 in the interior of Water Conservation Area 2A (WCA-2A-217) (26° 17.17' N, 80° 24.73' W ± 30 m) of the Florida Evergaldes. The hydrology of WCA-2A is managed to provide water supply during the dry season and water storage during the rainy season. Nutrient laded water, resulting from drainage of the Everglades Agricultural Area (EAA), is routinely pumped into WCA-2A across its northern border. Steady external nutrient loading has resulted in soil and water column P concentration gradient, with higher P concentrations near inflow structures and background P contents at interior sites (SWIM, 1992; Koch and Reddy, 1992: DeBusk et al., 1994). The unimpacted interior of WCA-

2A is characterized by peat soils, patchy sawgrass stands (*Cladium jamaicense* Crantz.), and abundant periphyton growth. The periphyton at WCA-2A-217 is dominated by calcareous blue-green algae (cyanobacteria), such as *Schizothrix calcicola* (Ag.) Gom. and *Scytonema hofmannii* Ag., as well as hardwater diatoms (Swift and Nicholas, 1987). This calcareous periphyton exists as as a layer on the soil surface, as coatings on the submerged portions of older stems of sawgrass, and occasionally forming floating mats on the water surface.

Field Experiments

Eight cylinders were formed from 1.6 mm thick clear polycarbonate sheets with dimensions of 122 cm tall by 56.5 cm diameter. These cylinders were inserted into the soil on September 5, 1995, to serve as experimental enclosures. Circular vents covered with mosquito netting approximately halfway up the cylinder allowed water exchange but reduced periphyton influx. Four enclosures had intact BP layers (+BP), while four had the BP removed (-BP) by suction provided by means of a hand operated diaphragm bilge pump. A Hydrolab (DataSonde 3, Hydrolab Corp., Austin, TX) was positioned 10 cm above the periphyton in one of the BP containing enclosures and recorded hourly measurements of water column temperature, dissolved oxygen (DO, percent saturation), pH, and electrical conductivity (EC). After 2 weeks, the Hydrolab was moved to a -BP enclosure (10 cm above soil surface) and recorded conditions for twelve more days.

Dissolved ions in porewater

Two weeks after insertion of the enclosures, Plexiglas pore water equilibrators (Hesslein, 1976) were installed in each of the enclosures. Cells of the pore water equilibrators were initially filled with deoxygenated (N_2 gas purging) distilled water and covered with 0.2 µm Nucleopore polycarbonate membrane (Nucleopore Corp., Pleasenton, CA) and a protective layer of 1 µm Spectra mesh nylon membrane cover

(Spectrum Corp., Los Angeles, CA). It is assumed that the concentrations of soluble ions in the soil porewater will equilibrate with the water in the equilibrator cells (Hesslein, 1976; Carignan, 1984). Pore water equilibrators were removed after 15 days incubation, the cells were emptied with syringes, and the water samples were analyzed for DRP (Ascorbic Acid Method 4500-P-E, APHA, 1992) on an AutoAnalyzer II (Technicon Corp.), pH, soluble Ca, and soluble Mg (by flame atomic absorption (FAA) (Method 3111, APHA, 1992). After these initial analyses, the remaining sample solutions were combined by depth (usually 5 cm increments). These samples were analyzed for total dissolved C (TDC), dissolved inorganic C (DIC), dissolved organic C (DOC) on a Rosemount Analytical Inc. DC-190 High-Temperature TOC Analyzer (Rosemount Analytical Inc. Dohrmann Division, Santa Clara, CA). SO_4-S and NO_3-N were analyzed by ion chromatography using a Dionex 4500i (Dionex Corp. Sunnyvale, CA.)(Method 4110, APHA, 1992). The ions K, Na, Cl, Si, Zn, Cu, Mn, Al, and Fe were analyzed by Inductively Coupled Argon Plasma Spectrometry (ICP) (Method 3120B, APHA, 1992).

In-situ phosphorus uptake

Lexan cylinders (14.5 cm I.D x 122 cm height) were inserted into the enclosures isolating an approximate 9.1 L volume of water above the BP layer in the BP containing enclosures, or a 10.7 L volume of water above the soil for the BP-less enclosures. Uptake of DRP by BP or soil was determined by depletion in the water column. Phosphorus as KH_2PO_4 was added to each of the cores to increase the ambient DRP concentration to by a nominal 100 $\mu g \ P \ L^{-1}$. Samples were collected 15, 30, 60, 90, 120, and 180 minutes after spiking, 0.2 μm filtered (Cameo acetate membrane, MSI Inc.) and stored in ice water or refrigerated at 4°C until analyzed for DRP. Additionally, a sample from each core was collected after 24 h to determine whether concentrations returned to ambient.

Greenhouse and Laboratory Experiments

Intact cores were collected on September 18, 1995. Twelve acrylic cylinders (14.5 cm I.D. x 47 cm height) were inserted through BP and into the soil to a 15 cm depth. Core insertion was made easier by cutting along the bottom edge of the cylinder with a serrated knife as the cylinders were inserted into the peat soil. Measurements were made to insure negligible compaction. The cores were transported upright to a greenhouse at the University of Florida. Bulk water was collected to replace that used during experimentation.

The intact cores were immersed to about 3/4 their height in a large water bath to reduce temperature fluctuations. Packing foam "peanuts" were floated on the water surface to reduce light penetration into the cores from directions other than from above. The BP layer was removed by suction in four of the cores to provide replicates for the -BP treatment. Bulk water was returned to these cores to replace the water removed during BP removal. The BP depth in the four replicates for +BP treatments was 7.3 ± 0.15 cm (mean ± SE). The water depth was set to 20 cm above the soil/water interface or above the BP/water interface. Air was continually bubbled, by an aquarium pump, into the surface water of all cores to provide aeration and to cause slight stirring.

Diel ambient DRP flux

The diurnal flux of DRP was investigated in eight cores, four of each treatment (+BP and -BP). This experiment was initiated 48 h after BP layers were removed. The pH was monitored at several locations in one core of each treatment. Small-tipped pH electrodes were installed in the core without periphyton at the soil/water interface and in the water column about 5 cm above the soil. The +BP core had pH electrodes installed at the soil/BP interface, at the BP/water interface, and in the water column about 5 cm above the BP. Ambient air temperature, water temperature, and incident radiation were also monitored. Air and water temperature varied 21 - 36° C, and incident radiation ranged 0 -

50 W m^{-2}. All cores had 20 cm depth of water providing a total water volume per core of 3.3 L. Water samples (10 ml) were collected mid-depth in each core once per hour for a 24 h cycle. Upon collection, water samples were immediately 0.2 µm filtered (Gelman Supor, Gelman Inc.) and stored on ice water or refrigerated at 4^{0} C until colorimetric analysis for DRP.

Fate of water column P

This experiment was conducted to determine the P exchange between the water column and underlying substrate; either benthic periphyton covered soil or soil alone, under increased water column P concentrations. Initial 10 ml water samples were collected immediately prior to the first spiking event. Each core then received 330 µg P as KH_2PO_4 by 10 ml addition to elevate the DRP concentration by 100 µg L^{-1}. The aerated water column was gently agitated to provide mixing. Water column DRP concentrations were sampled at time 0 (just before spiking),and approximately 15, 30, 60, 120, 180, 240, 300, and 360 min after spiking (the last sample collected per spiking event became the 0 min sample for the next spiking event). The water removed during sampling was replaced after collection of the last sample in each spiking event. Four spiking events occurred sequentially for a 24 h period starting at 09:00 on September 24, 1995. Sequential spikings occurred at 14:00 and 21:00 on September 24, and at 02:00 on September 25, 1995. This scheme provided for two uptake periods under light conditions (09:00 and 14:00) and two under dark conditions (21:00 and 02:00). Samples were 0.2 µm filtered and analyzed for DRP as above.

Approximately 24 h after the conclusion of the above experiment the four BP containing cylinders were sub-cored to provide 16 small cores with internal diameter of 4.5 cm. The 16 small cores were then randomly distributed into two treatments; those that would receive spiking with only ^{31}P as KH_2PO_4, and those that would receive ^{31}P and ^{32}P spiking. The ^{32}P was carrier-free H_3PO_4 in 0.02 *M* HCl (DuPont NEN). Cores were initially spiked to a total specific activity of 2.1 MBq L^{-1}. These treatments were further divided into those incubated under light conditions and those under dark. Using ^{32}P necessitated that this experiment be conducted under a light booth (providing 10 W m^{-2}) in the laboratory. All cores were submerged to approximately 3/4 their height in a water bath. Cores incubated under dark conditions were completely covered with aluminum foil. Cores used in the light treatment were covered to just below the periphyton layer to eliminate light penetration from the core sides. The water columns were slowly aerated by aquarium pumps. Temperature averaged 27 ± 4° C (mean ± SE).

Cores were spiked with KH_2PO_4 to an initial water column concentration of 110 µg P L^{-1}. Eight cores, four light and four dark, were then spiked with ^{32}P . Solutions were gently stirred and a 5 ml sample was collected to determine initial activity. Samples containing isotope were 0.2 µm filtered (Cameo acetate membrane disposable filters, MSI Inc.) and a 100 µL aliquot of the filtrate was added to 10 ml of scintillation cocktail (Scinti-Verse I, Fisher Scientific). Radioactivity was determined by counting for 5 min on a Beckman liquid scintillation counter (LSC) (LS3801, Beckman Instruments Inc.). Quench was insignificant and counting efficiency was approximately 100%. Water was collected from the non-radioactive cores and analyzed for DRP and from the isotope containing cores and analyzed for activity by LSC after 12 h incubations. Benthic periphyton was removed from all cores, filtered by gravity on Whatman #41 filter paper and weighted for total wet weight. Subsamples of the non-labeled BP were oven dried (70^{o} for 72 h) to determine water content.

Benthic periphyton from ^{32}P treatments was homogenized and a known freshweight subsample was extracted with 0.01 *M* HCl in a 1:100 solid to solution ratio for 2 h. This technique was proven as a satisfactory extraction technique to remove abiotically bound P in a previous study (see Chapter 3). Following extraction, a subsample was filtered and analyzed by LSC. The extracted BP and remaining 0.01 *M* HCl solution were digested at 100° C in a mixture of HNO_3 and H_2O_2 (modified Method

3030 E, APHA, 1992). After digestion a subsample was analyzed by LSC to determine total activity in BP. The difference between the initial water activity and the final water activity should equal that found in BP by digestion. Activity in extracted solution was considered ^{32}P abiotically bound to the $CaCO_3$ matrix of the periphyton and is a proportion of the total BP activity. The difference between abiotically bound activity and total activity is that biotically incorporated into the periphyton.

Statistical Methods

All statistical analyses were performed on SAS Proprietary Software Release 6.08 (SAS Institute Inc. 1989, Cary NC.). Where appropriate, the level of significance (p-value) is designated (p-value; *** = 0.001, ** = 0.01, * = 0.05, ns > 0.05). Correlation analysis was performed on the characteristics of the water column as measured by a Hydrolab, and on water depth measurements (SFWMD data) during the field enclosure study. The correlation coefficients (r) and the p-values are given for important relationships. Data resulting from detailed sampling of the pore water equilibrators (DRP, pH, Ca, Mg) were analyzed to determine significant effects of the presence of the BP layer. Data were treated as a Repeated Measures in Space analyzed as a Split Plot Design. The analysis of variance was modeled to determine the affects of depth, treatment (+BP and -BP), and the interaction.

Analysis of P uptake data under field and greenhouse conditions was accomplished by using a nonlinear regression procedure to fit the depletion data to an equation of the form

$$C = Co\, e^{-kt} + b \qquad [4\text{-}1]$$

where C = DRP concentration in solution at any time (t), Co is an estimate of the initial DRP concentration, k is the uptake coefficient (min^{-1}), and b is the theoretical, asymptotic equilibrium concentration. The nonlinear regression analysis used least squares criteria to obtain estimates of k; standard error (SE) estimates around k were determined using asymptotic theory for maximum and minimum likelyhood. The SE were then used to compare ks for curves from different treatments using a Z-test. The form of the Z-test is:

$$Z = (X_{trt1} - X_{trt2})/(\sigma_{trt1} + \sigma_{trt2})^{1/2} \qquad [4\text{-}2]$$

where X is the estimated value of k, and σtrti is the estimated SE of Xtrti. Resultant Z values were compared to the quantiles of normal distribution to determine the significance of the comparison. Nonlinear regression, as performed here , assumes observations are independent. This assumption is not strictly met in the experimental design used; therefore, the probability of committing a type I error is > 0.05, which means we may be declaring a comparison different when they really are not. Where appropriate, the initial uptake rates and comparisons of final concentrations were determined to be significantly different ($\alpha = 0.05$) by Student's t-test.

Results

Physicochemical Properties of the Water Column

Water temperature, in the field, varied between 27 - 32 °C with an average of 30 °C during the period September 5 - 30, 1995. The temperature showed diel variation with daytime temperatures usually 1-2 °C higher than at night (Fig. 4-1). The pH ranged 8.4 - 9.3 and varied within a 24 h period, although a distinct diel variation was not easily discernible (Fig. 4-2). The DO varied 33 - 75% saturation with an average of 48%. Considerable difference was observed in DO measured in the +BP enclosure versus that of the -BP enclosure (Fig. 4-3). The -BP enclosure had recorded min, max, and mean DO measurements of 33, 58, and 44% saturation, respectively, while those in the +BP

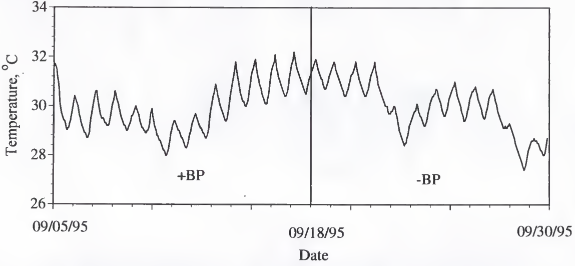

Fig. 4-1. Temperature recorded hourly with a Hydrolab installed in one of four replicate enclosures installed in WCA-2A-217. Hydrolab was installed in an enclosure with intact benthic periphyton (+BP) from September 5 thru 18, 1995. Hydrolab was then installed in an enclosure with BP layer removed (-BP) from September 18 thru 30, 1995.

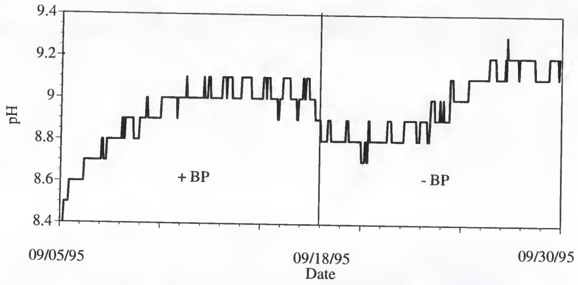

Fig. 4-2. pH recorded hourly with a Hydrolab installed in one of four replicate enclosures installed in WCA-2A-217. Hydrolab was installed in an enclosure with intact benthic periphyton (+BP) from September 5 thru 18, 1995. Hydrolab was then installed in an enclosure with BP layer removed (-BP) from September 18 thru 30, 1995.

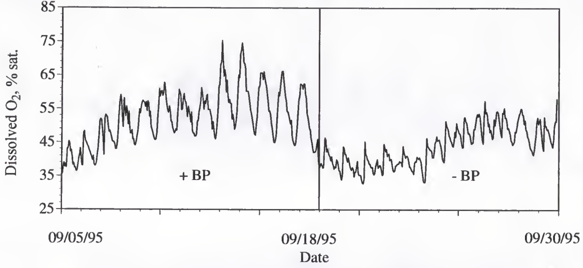

Fig. 4-3. Dissolved oxygen (DO) recorded hourly with a Hydrolab installed in one of four replicate enclosures installed in WCA-2A-217. Hydrolab was installed in an enclosure with intact benthic periphyton (+BP) from September 5 thru 18, 1995. Hydrolab was then installed in an enclosure with BP layer removed (-BP) from September 18 thru 30, 1995.

enclosure had respective values of 36, 75, and 52% saturation. The diel variation in DO in the +BP enclosure was generally between 20-30%, whereas that in the -BP enclosure was usually less than 10% (Fig. 4-3). Electrical conductivity ranged 97 - 119 mS m^{-1}, with average of 108 mS m^{-1}. There was little diel variation in EC (Fig. 4-4). The EC steadily increased once the Hydrolab was moved to the -BP enclosure. Increasing EC atthis time could be due to a general reduction in the water depth causing a concentration of ions, an increase in the flux of ions out of the soil and into the water column when the BP layer is removed, or to a combination of these effects.

Hourly values were averaged to obtain mean daily values of water temperature, pH, DO, and EC. These measurements were correlated to daily water depth (SFWMD data). The water depth ranged 72 - 103 cm with an average of 94 cm during the field study. However, the water depth was approximately 1 m through September 20, 1995, and then steadily decreased to 72 cm over the next 10 days. Water depth was inversely correlated with EC ($r = -0.88^{***}$). A depth reduction of 30 cm translated into an approximately 20 % increase in EC from 98 to 119 mS m^{-1}, suggesting a concentration of ions with decreasing water depth. Electrical conductivity was also inversely correlated to DO ($r = - 57^{***}$). Other correlations were not significant.

Electrodes monitored the pH at several positions in representative cores of the +BP and -BP treatments under greenhouse conditions. The pH of the water column in both treatments averaged 8.6 (Fig. 4-5). The water pH in the +BP core ranged between 8.3 - 8.8 while that in the -BP core similarly ranged 8.2 - 8.8. The pH of the water above the BP layer was more variable than that in -BP core. The electrode positioned at the soil/water interface in the -BP treatment recorded pH levels very similar to that of the water, suggesting that when the BP layer is not present, the surface soil equilibrates to the water. The pH at this point averaged 8.5 and ranged 8.3 - 8.6. The pH recorded at the

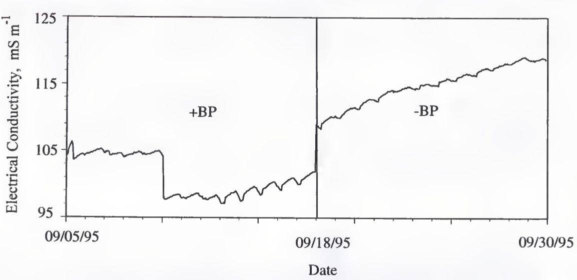

Fig. 4-4. Electrical conductivity (EC) recorded hourly with a Hydrolab installed in one of four replicate enclosures installed in WCA-2A-217. Hydrolab was installed in an enclosure with intact benthic periphyton (+BP) from September 5 thru 18, 1995. Hydrolab was then installed in an enclosure with BP layer removed (-BP) from September 18 thru 30, 1995.

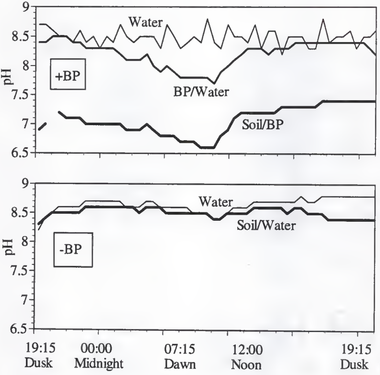

Fig. 4-5. Simultaneously recorded pH at several positions in intact cores incubated under greenhouse conditions September 21-22, 1995. Benthic periphyton was removed from one set of cores (-BP) while the other cores contained benthic periphyton layers (+BP). Electrodes were installed in the -BP cores at the soil/ water interface (Soil/Water) and about 5 cm above the soil/water interface (Water). Electrodes were installed in the +BP cores at the soil/bentic periphyton interface (below approximately 7 cm of BP) (Soil/BP), at the periphyton/water interface (BP/Water), and about 5 cm above periphyton in the water column (Water).

BP/water interface of the +BP treatment averaged 8.2 and ranged 7.7 - 8.5. The soil/BP layer had pH that averaged 7.1 and ranged 6.6 - 7.3. The pH at the BP/water and soil/BP interfaces were generally lower than at the soil/water interface of the -BP treatment.There was some diurnal variation evident in the pH values recorded at the BP/water and BP/soil interfaces of the +BP treatment. At these two points the pH was generally lowest between midnight and early morning and increased about 0.6 pH units in about 2 h to maximum in the afternoon.

Gradients in Dissolved P and other Ions

Dissolved reactive P in water above either the BP layer or the soil surface had a mean DRP concentration of 5 $\mu g\ L^{-1}$. The DRP in the water of the +BP treatment was slightly more variable than above the soil in the -BP treatment (Fig. 4-6). Minimum and maximum recorded DRP in the water of the +BP treatment was 4 and 10 $\mu g\ L^{-1}$ respectively, while that in the -BP treatment was 4 and 7 $\mu g\ L^{-1}$. The BP layer had mean DRP of 9 $\mu g\ L^{-1}$ and with a minimum of 4 $\mu g\ L^{-1}$ and a maximum of 16 $\mu g\ L^{-1}$. The DRP in the interstitial water of the BP surface was lower than at depth in the layer (Fig. 4-6); thus, the BP layer provides a transition from the DRP of the water to that of the soil. The presence of the BP layer causes a higher level of DRP to be maintained in the porewater of the soil. Soil porewater DRP ranged from 6 to 13 $\mu g\ L^{-1}$ with a mean of 9 $\mu g\ L^{-1}$ in the 0 to 15 cm deep soils under intact periphyton layers. The DRP in soils with the BP layer removed ranged 5 - 13 $\mu g\ L^{-1}$ with mean DRP of 7 $\mu g\ L^{-1}$. Although these ranges are similar, statistical analysis showed the DRP of the soil porewater to be significantly different between the two treatments (p value = 0.0473).

The pH (Fig. 4-7) in the water column was high and decreased with depth (p value = 0.0001). There was no significant difference in the pH between treatments suggesting that the presence or absence of a BP layer does not appreciably change the pH of the soil

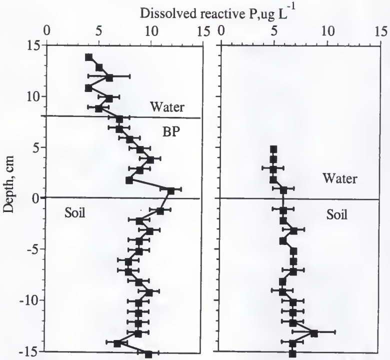

Fig. 4-6. Dissolved reactive phosphorus concentration (DRP) (Mean ± SE, n=4) with depth as determined by porewater equilibrators. Equilibrators were installed in field enclosures from September 18 to October 3, 1995. Enclosures contained intact benthic periphyton layers (left) or had periphyton removed (right).

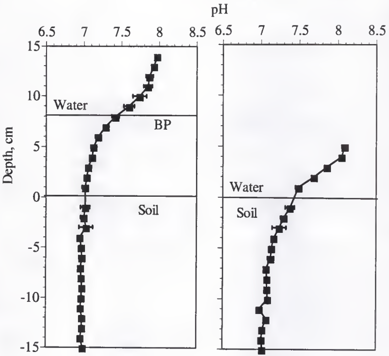

Fig. 4-7. pH (Mean ± SE, n=4) with depth as determined by porewater equilibrators. Equilibrators were installed in field enclosures from September 18 to October 3, 1995. Enclosures contained intact benthic periphyton layers (left) or had periphyton removed (right).

porewater. The steepest portion of the pH gradient occurred between the water layer and the first few cm of the BP layer. This transitional layer is seen as occurring between thewater and the first few cm of the soil in the -BP treatment. Benthic periphyton acts as a transition zone for pH changes between the soil and water when present (Fig. 4-7).

The Ca concentration of the water above the BP layer ranged 68 - 101 mg L^{-1} with mean concentration of 79 mg L^{-1}, while that in the water of the -BP treatment was in the range of 81 - 119 mg L^{-1} with mean concentration of 94 mg L^{-1} . The Ca concentration of the BP interstitial water ranged 82 -132 mg L^{-1} . The mean Ca concentration of the soil porewater under the BP layer was 127 mg L^{-1} and showed very little variation with depth, which was significantly higher than the Ca content of the soil porewater in the -BP treatment ranged (p value = 0.0045; Fig. 4-8). There was no significant difference in the Ca concentration of soil porewater with depth in either treatment. Although not statistically significant, the Ca concentration in the soil porewater tends to increase with depth in the -BP treatment.

The Mg content of the water column was similar for both treatments and was approximately in the same range as soil porewater (Fig. 4-9). The Mg contents of the surficial BP layers were lower than at the BP/soil interface (Fig. 4-9). Like the Ca contents, the Mg contents of the +BP porewater were significantly higher than the -BP soil porewater (p value = 0.0056). The porewater of the +BP treatment ranged 11 - 14 μg L^{-1} with mean of 13 mg L^{-1} . In the -BP soil porewater the Mg contents ranged 10 -13 μg L^{-1} with a mean of 11 mg L^{-1} . Unlike the Ca profiles, there was a significant effect of depth on the Mg contents of the soil porewater for each of the treatments. The porewater Mg contents increased with soil depth for each treatment (p value = 0.0001).

The dissolved inorganic C (DIC) was significantly higher in the 0-5 cm layer when BP is present than when removed (Table 4-1). There was not a corresponding difference in the dissolved organic C (DOC) contents of the two treatments. These results are consistent with those previously observed for dissolved Ca (Fig. 4-8). The

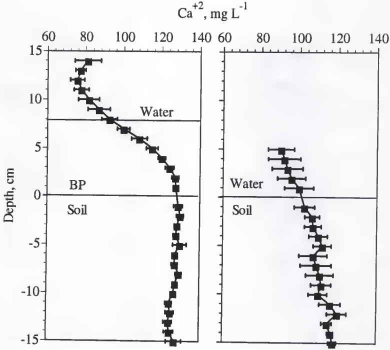

Fig. 4-8. Calcium concentrations (Mean ± SE, n=4) with depth as determined by porewater equilibrators. Equilibrators were installed in field from September 18 to October 3, 1995. Enclosures contained intact benthic periphyton layers (left) or had periphyton removed (right).

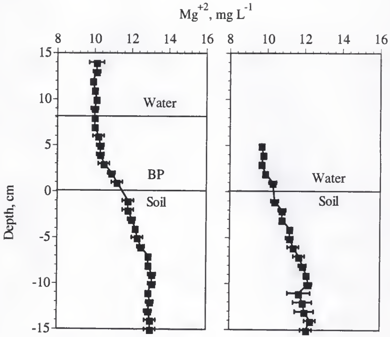

Fig. 4-9. Magnesium concentrations (Mean ± SE, n=4) with depth as determined by porewater equilibrators. Equilibrators were installed in field from September 18 to October 3, 1995. Enclosures contained intact benthic periphyton layers (left) or had periphyton removed (right).

Table 4-1. Chemical characteristics of water column, interstitial water from the benthic periphyton layer (BP) (thickness = 8.3 ± 0.4 cm), and soil porewater at three depths below the soil/water column interface. Water was collected from porewater equilibrators incubated in WCA-2A-217 field enclosures from September 18 to October 3, 1995. Enclosures contained intact BP (+BP) layers or had BP removed (-BP). Porewater equilibrators collected water samples at 1 cm increments. These samples we composited within each layer to provide adequate sample for anaysis. (Mean ± SE, n = 4 unless noted † then n = 3, or ‡ then n = 2). (Zn, Cu, Mn, Al, and Fe, also analyzed <0.01 mg L^{-1}). Results for the two treatments (+BP and -BP) were compared by seperation of means analysis for each layer. Differences are given after the values for the +BP treatment. Thus, designations following a value compare that value in the +BP treatment to the corresponding value in the -BP treatment. (p values; ** = 0.01, * = 0.05, ns = >0.05).

Parameter	+BP					-BP			
			Soil (depth, cm)				Soil (depth, cm)		
mg L^{-1}	Water	BP	0-5	6-10	11-15	Water	0-5	6-10	11-15
TDC	110 ± 2^{ns}	145 ± 7	$158 \pm 7^{*}$	162 ± 4^{ns}	153 ± 6^{ns}	112 ± 4	136 ± 4	141 ± 10	152 ± 3
DIC	58 ± 4^{ns}	87 ± 4	$108 \pm 4^{*}$	109 ± 4^{ns}	$104 \pm 4^{*}$	70 ± 10	84 ± 5	97 ± 7	93 ± 2
DOC	52 ± 3^{ns}	58 ± 3	50 ± 7^{ns}	52 ± 2^{ns}	$50 \pm 2^{*}$	51 ± 3	51 ± 4	44 ± 10	59 ± 2
K	11.3 ± 0.7^{ns}	11.4 ± 0.4	$13.1 \pm 0.7^{**}$	13.4 ± 0.6^{ns}	$13.0 \pm 0.6^{*}$	10.3 ± 0.3	11.1 ± 0.5	11.6 ± 0.6	10.9 ± 0.5
Na	111 ± 1^{ns}	108 ± 2	122 ± 5^{ns}	133 ± 6^{ns}	139 ± 4^{ns}	112 ± 1	119 ± 4	129 ± 4	133 ± 3
Cl	148 ± 1^{ns}	162 ± 8	164 ± 8^{ns}	174 ± 9^{ns}	187 ± 8^{ns}	148 ± 2	158 ± 5	170 ± 6	184 ± 8
Si	12.6 ± 0.3^{ns}	18.8 ± 0.7	$20.7 \pm 0.9^{**}$	$19.3 \pm 1.3^{*}$	17.7 ± 1.3^{ns}	12.1 ± 0.3	14.5 ± 0.7	15.0 ± 0.6	14.5 ± 0.4
SO_4	17.2 ± 0.4^{ns}	7.6 ± 0.9	$6.1 \pm 0.7^{**}$	5.8 ± 0.9^{ns}	6.3 ± 2.1^{ns}	16.3 ± 0.8	9.9 ± 0.7	7.1 ± 0.7	5.1 ± 0.4

presence of BP maintains higher Ca and DIC concentrations (as $CaCO_3$) in the interstitial water then when not present. The DIC between treatments was not significantly different at the 6-10 cm soil depth but was at the 11-15 cm depth. In all soil depths the DIC in the +BP treatment is higher than in the -BP; however, the variability in the 6 - 10 cm layer is such that differences were not statistically significant. Similarly, the K concentrations are generally higher under BP than without BP. The Si content of the porewater is higher under BP than in the -BP treatment. The BP is partially composed of diatoms which have silaceous tests; therefore, the presence of diatoms in BP may increase the Si activity. There was no significant difference in Si content at the lower soil depth probably due to diminished BP influence. The SO_4 content of the 0-5 cm soil layer is significantly lower under BP than when the BP is not present. This suggests a lower state of oxidation at the soil surface when BP is present. This is due to increased demand for O_2 by the heterotrophic component of the periphyton.

There was no diel variation in the DRP concentration of the water column in the cores with or without BP (Fig. 4-10). Occasionally a sample was obtained with a high DRP level causing variation, but this was attributed to contamination as there was no distinct trend with time or between cores of the same treatment. The ambient water concentration was only 4 $\mu g\ L^{-1}$. This concentration is low and is similar to that often found in the field.

The in situ DRP profiles suggest that some soil P should have been released from the porewater into the water column when the BP layer was removed. However, this release did not result in increased DRP levels in the water column.

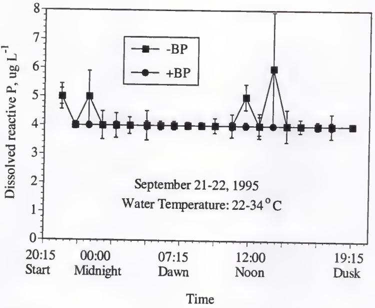

Fig. 4-10. Dissolved reactive phosphorus (DRP) concentration in the water column of intact soil cores incubated under greenhouse conditions. Benthic periphyton was removed from one set of cores (-BP) while other cores had a 7 cm benthic periphyton layer (+BP).

Phosphorus Removal from the Water Column

In situ experiment

The depletion of DRP from the water column generally followed first-order reaction kinetics (Fig. 4-11). The P removal rate constants under field conditions were 0.031 ± 0.005 and 0.023 ± 0.005 min^{-1} for the +BP and -BP treatments, respectively, and the k values between treatment were not significantly different. The mean DRP concentrations remaining in the water after 3 h was 51 ± 4 and 80 ± 4 $\mu g\ P\ L^{-1}$ for the +BP and -BP treatments, respectively, which were significantly different (p value = 0.0018). Initial uptake rates were calculated for the 15 min period between the first and second timed sampling. Since the first sampling was delayed to about 15 minutes after initial spiking, the actual initial uptake rate is probably slightly greater than calculated. The initial uptake rates are expressed in terms of the surface area of either the BP layer or the soil exposed (165 cm^2). Mean initial uptake rates (± SE) were 34 ± 13 $\mu mol\ P\ m^{-2}\ min^{-1}$ for the +BP treatment and 22 ± 6 $\mu mol\ P\ m^{-2}\ min^{-1}$ for the -BP treatment. The initial uptake rates were not significantly different. These results show that even though the uptake rates are not significantly different between the treatments, the cylinders with intact BP layers were able to remove substantially more DRP from the water column than the treatment without BP. After 24 h the DRP in the +BP treatment approximately equal to ambient water concentration (5 $\mu g\ L^{-1}$) while that in the -BP treatment was significantly greater at 18 ± 6 $\mu g\ L^{-1}$. (p value = 0.0397).

Greenhouse experiment

Similar P depletion from the water column was also observed for soil cores incubated under greenhouse conditions (Fig. 4-12; Table 4-2).

Removal of DRP was faster in the -BP treatment than in the +BP treatment during the spiking event that began at 09:00. The initial uptake rate and the rate constant

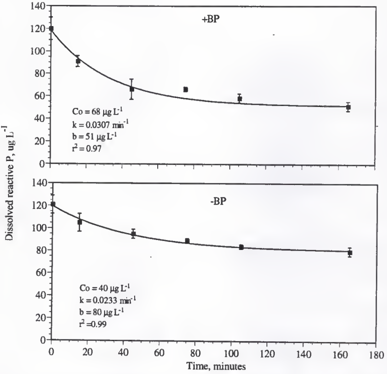

Fig. 4-11. Dissolved reactive P (DRP) concentration (Mean ± SE, n = 4) in the water column during field uptake studies conducted in WCA-2A-217 on October 2, 1995. Lexan cylinders were inserted into enclosures with intact benthic periphyton layers (+BP, upper) or with the BP layers removed (-BP, lower). Depletion of spiked P (as KH_2PO_4) was modeled using the equation $C = Co\ e^{-kt} + b$.

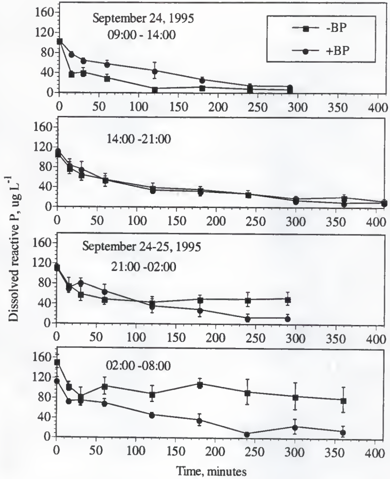

Fig. 4-12. Changes in dissolved reactive P (DRP) concentration in the water column of intact cores incubated under greenhouse conditions with benthic periphyton intact (+BP) or with the BP layer removed (-BP). Phosphorus removal from the water was determined by sequentially spiking the water column at 100 µg L^{-1} above ambient on September 24-25, 1995.

Table 4-2. Initial uptake rates, initial concentrations (Co), rate constants (k), final concentrations (b), and r^2 for intact core P uptake experiments conducted under greenhouse conditions on September 24-25, 1995. Phosphorus depletion from the water column was measured in cores with benthic periphyton layers present (+BP) or with periphyton layer removed (-BP). Uptake was determined under light (09:00 -14:00 and 14:00 - 21:00) and dark (21:00 - 02:00 and 02:00 - 08:00) conditions. Initial uptake rates were calculated for P depleted in the first 15 min. Rate constants and r^2 result from fitting a first-order equations of the form $C = Co\ e^{-kt} + b$ to depletion data.

Experiment Start - Finish Time	Initial Uptake Rate Mean ± SE	Initial Concentration (Co)	First-order Rate constant (k)	Final Concentration (b)	r^2
	μmol m^{-2} min^{-1}	μg P L^{-1}	min^{-1}	μg P L^{-1}	
09:00 -14:00					
+BP	11 ± 1.9	105	0.0128	14	0.95
-BP	29 ± 2.0	105	0.0461	7	0.92
14:00 - 21:00					
+BP	13 ± 5.4	114	0.0127	10	0.97
-BP	12 ± 5.1	107	0.0123	13	0.96
21:00 - 02:00					
+BP	17 ± 2.6	110	0.0127	14	0.93
-BP	16 ± 6.9	113	0.0067	52	0.97
02:00 - 08:00					
+BP	25 ± 1.0	114	0.0120	16	0.91
-BP	21 ± 4.0	152	0.0964	80	0.73

were significantly higher for the -BP treatment than for the +BP treatment (p-value = 0.0008). The final DRP level of the water column after 290 minutes was 14 $\mu g\ L^{-1}$ for +BP and 7 $\mu g\ L^{-1}$ for -BP, which were significantly different (p value = 0.0205; Table 4-2).

Uptake of DRP was nearly identical for both treatments during the 1400 spiking event (Fig. 4-12). Initial uptake rates were not statistically different for this or the remaining two spiking events. The initial concentrations, first-order rate constants, final concentrations after 410 minutes, and r^2 values resulting from this event are all similar for the two treatments suggesting similar P uptake (Table 4-2). The final concentrations were not statistically different at the end of this spiking event.

Although the initial uptake rates were similar, with the spiking event beginning at 21:00, the -BP cores lost the ability to remove P from the water column. Within the 290 minutes of this experiment, the -BP cores removed around 60 $\mu g\ P\ L^{-1}$, whereas the +BP cores removed an average 96 $\mu g\ L^{-1}$. Final solution concentrations were different between the treatments, with -BP being significantly higher than +BP (p value = 0.0413; Table 4-2).

The diminishing ability of the -BP cores to remove DRP from the water column becomes more apparent during the final (02:00) spiking event. The -BP first-order rate constant is much greater than that of the +BP treatment. This does not suggest that the uptake rate is greater in the -BP than the +BP treatment, but only that the -BP treatment reaches an asymptotic water column concentration more rapidly than the +BP treatment. The increased variability in the samples at the end of the experiment also manifest as relatively low r^2 values. This is especially apparent for the -BP treatment. The average final concentrations attained for the two treatments were 16 ± 11 for the +BP treatment and 80 ± 25 $\mu g\ L^{-1}$ for the -BP treatment (Table 4-2). This variability is due to one anomalous core in each treatment and results in the final concentrations that were not statistically different. Removing the anomalous core from each treatment (n = 3), the final DRP concentration of the two treatments becomes 4.6 ± 0.3 μg L^{-1} for +BP and 104 ± 12 μg L^{-1} which are highly significantly different (p value = 0.0002). Over the course of four spiking events the +BP treatment removed DRP on an areal basis of 7.0 μg cm^{-2} while that of the -BP treatment removed 5.8 μg cm^{-2}.

Phosphorus depletion from the water column was not affected by light/dark conditions, as the differences in uptake rates were not significant. Likewise, the final water column DRP concentrations in the +BP treatment were not different between the four runs..

Fate of Phosphorus Under Light and Dark Conditions

Partitioning of P removed from the water column by BP was determined using ^{31}P and ^{32}P spiked floodwater in cores incubated under light and dark conditions for 12 h. Floodwater initially contained 110 μg P L^{-1} . At the end of 12 h an average of 60 % of the spiked P was removed from the water column under light conditions while an average of 45 % was removed under dark incubation (Table 4-3.). Means comparison by t-test show these results were not significantly different. A total mass removal 1.29 μg P cm^{-2} was removed under light conditions, and 0.97 μg P cm^{-2} was removed under dark conditions during 12 h of incubation.

The P levels in the water column of the BP containing treatment returned to an average of about 16 μg L^{-1} at the end of the previous series of experiments. The final DRP concentration at the end this experiment was between 44 and 60 μg L^{-1} . This may indicate that the P uptake capacity of the BP layers is becoming saturated due to P loading. Phosphorus uptake per gram dried BP seemed greater under light conditions however variability in the samples caused a nonsignificant difference. Similarly, the abiotically bound P on the BP surfaces (see Chapter 3), was not significantly different between the light and dark treatments.

Table. 4-3. Results of small core laboratory uptake experiment conducted September 27, 1995. ^{31}P initial concentration in solution = 110 $\mu g\ L^{-1}$ based on water height of 20 cm above BP layer (water volume = 310 ml). Floodwater sampled after 12 h. BP subsample extracted with 0.01 *M* HCl.

Reps	Final DRP conc. 12 hour	DRP removed from solution per g dw BP	0.01 *M* HCl extracted DRP per g dw BP
	$\mu g\ L^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$
Light			
1A	37	9.7	44.2
2A	58	13.2	22.8
3A	42	8.1	31.4
4B	37	15.1	25.6
Mean ± SE	44 ± 5	11.5 ± 1.6	31.0 ± 4.8
Dark			
1C	46	7.3	39.1
2C	71	6.2	53.4
3C	47	7.8	31.0
4A	76	8.8	69.4
Mean ± SE	60 ± 8	7.5 ± 0.6	48.2 ± 8.4

Approximately 72% of total ^{32}P activity removed from solution was recovered in the digestion. Recoveries between replicates were highly variable (Table 4-4). The activities recovered in the HCl extraction were expressed as a percentage of total digested activity recovered. These data suggest that under the conditions of these experiments about 14% of the P removed from the water column is in the abiotic portion of the periphyton, while the remaining 86% is biotically incorporated.

Disscussion

The photosynthetic activity of periphyton is the primary source of oxygen in open water areas and for most of the Everglades ecosystem (Rader and Richardson, 1992; Belanger et al., 1989). The spatial and temporal variations in oxygen content of the water column are largely influenced by those factors that influence the photosynthetic activity of the periphyton. The oxygen status of the water column fluctuated diurnally, with greatest percent saturation at mid-day (Fig. 4-3), but remained oxic throughout the diurnal cycle. Proximity to the photosynthetic surface of the periphyton influences oxygen status. Spatial variation occurred, with the oxygen status of water overlying BP being greater than that without periphyton. In unimpacted Everglades sites, Rader and Richardson (1992) showed DO at the periphyton/water interface varied diurnally between 3 - 30 mg L^{-1} while the soil/periphyton interface remained anaerobic throughout the 24 h period. Similarly, oxygen profiles, measured during the day in intact BP-containing cores showed rapid depletion within one cm below the periphyton/water interface resulting in anoxic conditions at the soil/periphyton interface (see Fig. 5-1). Conversely, Carlton and Wetzel (1988) found lake sediment surfaces covered with periphtyon were oxidized during the day and anoxic at night. The periphyton layers in their study were generally a few millimeters thick. The balance between the production and consumption of oxygen

Table 4-4. Results of small core laboratory uptake experiment conducted September 27, 1995. ^{31}P initial concentration = 110 $\mu g\ L^{-1}$. Cores were simultaneously spiked with ^{32}P. Floodwater was sampled for activity just after spiking (< 5 min) and at 12 h. Benthic periphyton (BP) was extracted with 0.01 *M* HCl and then digested to determine HCl-extractable-P and total periphyton incorporated P, respectively. One replicate of the dark incubation was omitted as an outlier.

Reps	Total Activity Removed from Solution	Total 0.01 M HCl Extractable Activity	Total Digested Activity	Percent Total Activity Recovered in Digest	Extractable Activity as Percent of Digest Activity
	cpm	cpm	cpm	%	%
Light					
1D	2.43×10^7	2.62×10^6	2.27×10^7	93.6	11.5
2B	2.25×10^7	1.47×10^6	1.14×10^7	50.7	12.9
3B	2.02×10^7	2.60×10^6	1.44×10^7	71.0	18.1
4C	2.06×10^7	2.07×10^6	1.60×10^7	77.4	13.0
Mean ±	2.19×10^7±	2.19×10^6±	1.61×10^7 ±	73.2 ±	13.9 ±
SE	9.32×10^5	2.73×10^5	2.40×10^6	8.9	1.5
Dark					
1B	2.05×10^7	4.25×10^6	1.78×10^7	86.7	23.8
2D	3.25×10^7	2.07×10^6	1.51×10^7	46.7	13.7
3D	3.74×10^7	1.86×10^6	3.04×10^7	81.4	6.1
Mean ±	3.01×10^7 ±	2.72×10^6 ±	2.11×10^7 ±	71.6 ±	14.5 ±
SE	5.00×10^6	7.64×10^5	4.71×10^6	12.5	5.1

determines the oxygen status. If periphyton layers are thin, such as in the Carlton and Wetzel (1988) study, the daytime photosynthetic O_2 production exceeds the biological and chemical oxygen demand of the periphyton and surficial sediments. In this study microbial respiration within the lower portions of thick BP (> 7 cm) layers exceeded the oxygen produced. Similar results were also reported by Rader and Richardson (1992).

The pH was similarly influenced by BP activity, with pH above 8.5 in the water column but around 7 at the soil/BP interface. Rader and Richardson (1992) measured pH values ranging from 7.5 - 10 at periphyton/water interface with maximum occurring during peak photosynthesis while the pH of the water column and soil/periphyton was 7.5 - 8.0.

The in situ DRP profiles of the soil-water column suggest a concentration gradient between the water column and the interstitial water of the BP and soil. The -BP treatment shows that soil porewater reached a new "equilibrium" concentration with the surface water. Similar trends were also seen with the Ca concentrations. Although the mechanism is not known, BP maintains higher soil porewater DRP and Ca concentrations. The pH is lower at the soil/BP interface than at the soil/water interface in -BP cores suggesting that this phenomenon is not a function of the stability of Ca-P with high pH. An alternative hypothesis is that the $CaCO_3$-containing BP supports higher $CaCO_3$ equilibrium concentrations which in turn bind P through adsorption or coprecipitation. Using in situ DRP concentrations, the diffusive P-flux from the BP into the water column was calculated following Moore et al. (1991). Selecting representative positions in the water column and periphyton layer and the DRP concentrations at those positions, flux out of the BP layer into the water column was calculated to be 3.5 - 5.8 μg m^{-2} d^{-1}. This rate of P flux is lower than the 2.7 mg m^{-2} d^{-1} that was observed from sediments of a hypereutrophic Florida lake (Moore et al., 1991). Applying these calculated flux rates to the greenhouse core flux experiment would predict that DRP should have increased in the water column of the +BP treatment at a rate of 0.0007 -

0.0012 μg L^{-1} h^{-1}. Similar or greater flux should have been observed in the -BP cores. Raising the water column DRP concentration by this much is below detection with the analytical methods used. There was not a measurable increase in the water column DRP during the 24 h of the ambient flux experiment. A possible explanation is that the biota of the water column, periphyton, and soil is severely P limited; therefore, there is rapid assimilation of any available DRP. Samples were collected, filtered, and analyzed for DRP. It is not known whether an increase in the TP of the water column would have been observed.

Uptake rates on area basis were calculated using the linear portion of the depletion curves. Dissolved reactive P uptake during this study ranged between 200 - 1340 mg m^{-2} d^{-1} . Horner et al. (1990) estimated maximum uptake rates of 30 mg m^{-2} d^{-1} for periphyton grown on slides in experimental streams. Their study was conducted with thin layers of periphyton with maximum solution concentration of 50 μg L^{-1} , half that used in this study. Adey et al. (1993) determined maximum uptake to be 120 mg m^{-2} d^{-1} in systems designed to maximize P removal from water. Again, a thin layer of adnate periphyton was used with water P concentrations varying 12 - 148 μg L^{-1} . In experimental streams used to determine the P-flux in sediment, House et al. (1995) measured fluxes around 19 mg m^{-2} d^{-1} at unaugmented P concentrations (110 μg L^{-1}). When the influent P concentration was raised ca. 20 μM (620 μg L^{-1}), the flux rates increased to over 430 mg m^{-2} d^{-1}. Accretion rates based on ^{137}Cs dating for total P in the peat soils of this site were estimated to be 25 mg m^{-2} yr^{-1} (see Chapter 2). The areal uptake rates determined in this study are greater than those expected under ambient P concentrations. These experiments were conducted for short-term uptake, and it is unlikely that the system can sustain this high uptake continually. These experiments show the system saturates with continued loading. Prolonged enrichment would also change the composition of the system, as evidenced by the change in periphyton community structure observed by Swift and Nicholas (1987). Uptake on a long -term basis would be much lower, considering the asymptotic nature of the depletion curves. However, knowing the maximal uptake rates may be useful in designing periphyton treatment systems in which pulses of P enriched water is applied. Also interesting is the comparison of P uptake by systems with intact BP layers verses those without these layers. The periphyton treatment continued to remove P from the water column but the periphyton-less treatment began to saturate and lose the ability to remove P. In earlier studies by Davis (1982) at an unenriched Everglades site, 2-4% of added ^{32}P activity remained in the water of periphyton-containing enclosures after 10 days, while 16% remained in the water of enclosures where periphyton was absent.;

Phosphorus removal from the water column involves mechanisms that are biological, such as algal uptake, or physicochemical, such as adsorption and precipitation. Benthic periphyton is involved in both removal mechanisms, directly through uptake, and indirectly by creating physicochemical conditions for abiotic P removal.

Phosphorus uptake by periphyton is influenced by the P concentration in the water and by diffusion P to the periphyton surface (Loch and John, 1979). Numerous studies have been conducted to determine the influence of water velocity, boundary layer conditions, and nutrient diffusion on the supply of P to periphyton (Loch and John, 1979; Riber and Wetzel, 1987; Burkholder et al., 1990; Horner et al., 1990). Most of these studies were conducted on periphyton films, grown on natural or artificial substrates, that are a few millimeters thick. The BP of the Everglades is generally 4 - 10 cm thick and can be described as a loose, thick mat of filamentous cyanobacteria (Gleason and Spackman, 1974; Rader and Richardson, 1992; Browder et al., 1994) which makes comparisons between these studies difficult. As the thickness and the coherence of the mat increases, the recycling of nutrients within the mat becomes increasingly important (Sand-Jensen, 1983).

Numerous authors have suggested that the supply of P to the periphyton is limited by diffusion of P at the mat surface (Loch and John, 1979; Riber and Wetzel, 1987;

Burkholder et al., 1990; Horner et al., 1990). Phosphorus supply to the lower layers of periphyton occurs only after P has cycled through the surface material. The intensity of nutrient cycling within the periphyton is largely determined by the degree of limitation (Wetzel, 1993). Thick periphyton mats, with highly P-limited interior layers, may develop large P demands, thus increasing the gradient between the periphyton and the water column. This would subsequently increase the rate of diffusion. If the P concentration in the BP surface is assumed to average 7 $\mu g\ L^{-1}$ (Fig. 4-6), the enriched water column P concentration is assumed at 110 $\mu g\ L^{-1}$, and the diffusive distance between the water column and a minimal depth in the periphyton is 0.1 cm, then using Fick's first law of diffusion, as in Moore et al. (1991), the steady state diffusive flux calculates to be 6.7 mg $m^{-2}\ d^{-1}$. Calculating in this way should provide a maximum P flux from the water column into the periphyton. This calculated flux is orders of magnitude lower than the empirical uptake rates suggesting an uptake mechanism that greatly exceeds that solely by diffusion.

The benthic periphyton in the unenriched Everglades is composed largely of cyanobacteria, which readily precipitate $CaCO_3$ (Gleason, 1972; Browder et al., 1994). Calcification accompanies the photosynthetic utilization of bicarbonate in a reaction that can be summarized as

$$Ca^{2+} + 2HCO_3^- \Rightarrow CaCO_3 + CO_2 + H_2O \qquad [4\text{-}3]$$

The Everglades peat is underlain by calcareous bedrock that provides a continuous supply of bicarbonate ions (Rader and Richardson, 1992; Gleason and Spackman, 1974; Gleason, 1972). Utilizing bicarbonate allows the algal component of periphyton to continue photosynthesis after the water column CO_2 has been depleted, thus conferring an advantage over autotrophs that rely solely on CO_2. The precipitation of $CaCO_3$ can affect the P chemistry of the water column by coprecipitation. Coprecipitation can occur by two mechanisms, either P is adsorbed to the surface of previously formed $CaCO_3$ or P forms a mixed solid with precipitating $CaCO_3$ (Otsuki and Wetzel, 1972; House et al., 1995). The formation of mixed solids has been shown to remove more P and at a faster rate than adsorption (Otsuki and Wetzel, 1972). The reactions of P with $CaCO_3$ are greatest at pH > 9.0, a commonly observed phenomenon in Everglades surface water (Fig. 4-2), especially in close proximity to the periphyton surface (Rader and Richardson, 1992). Phosphorus has been shown to be abiotically bound to the $CaCO_3$ matrix of Everglades periphyton (see Chapter 3). Similarly, McConnaughy (1991) showed P precipitated on the external surfaces of $CaCO_3$ encrustations on algae cells (*Chara corallina*).

Phosphorus uptake by peat soils results from both physical adsorption and metabolic assimilation in microbes (Amador et al., 1992; Davis 1982). Microorganisms and small particles control the initial P uptake rates in wetland soils, especially during periods of low nutrient concentration (Richardson and Marshall, 1986). Amador et al. (1992) found initial rapid P uptake by Everglades peat soils was largely microbial. They also suggested P is abiotically retained by Ca-P precipitates (as above) or possibly by complexation of organic matter, Ca ions, and P to form phospho-humate complexes (Amador et al., 1992). Long-term P retention in peat wetlands is controlled by peat accumulation and soil adsorption (Richardson and Marshall, 1986). My research has shown that although the initial rate of P removal by peat soil is more rapid than uptake by BP the P retention capacity of soils rapidly decreases with continued loading. Similar results were obtained in studies comparing P retention in enriched and unenriched Everglades peat (Amador et al., 1992).

Abiotic and biotic mechanisms are responsible for the removal of DRP from the water column by BP with biological assimilation being the dominant mechanism. Biotic uptake was responsible for 86% of the ^{32}P removed from solution. Using living and dead (killed with formaldehyde treatment) periphyton covered slides, Loch and John (1979) found 50 - 75 % of P uptake was due to biological activity. They ascribe the remaining P uptake to adsorption by the "polysaccharide-like" matrix.

Phosphorus limitation was shown to increase the calcification in a marine coccolithophorid algae by more than 60% (Paasche and Brubak, 1994). If the opposite condition, that is, increases in P concentration cause calcifying organisms in the periphyton to halt $CaCO_3$ formation, then this may explain why $CaCO_3$ encrustations or calcifying periphyton is excluded from nutrient enriched water (Swift and Nicholas, 1987). Swift (1981, 1984), Swift and Nicholas (1987), and Hall and Rice (1990) all revealed a shift from blue-green algae species to green algae with N and P additions. Contrastingly, Vymazal et. al. (1994) found the number of blue-green (calcifying) algal species increased 100% in fertilized plots in the Everglades when compared to unfertilized controls. Consequences of the loss of the BP layer in the Everglades would manifest as a reduction in the short-term uptake of DRP. Although initial uptake rates are rapid for the uncovered soil, the retention capacity is quickly saturated. The BP also maintains higher Ca and P concentrations in the soil porewater. With loss of this layer, a flux of P from the soil to the water column is expected.

Conclusions

Benthic periphyton affects the physicochemical characteristics and the P chemistry of shallow waterbodies. Photosynthesis causes high levels of water column oxygen and diurnally fluctuating pH conditions. The precipitation of $CaCO_3$ by calcifying BP provides substrate for the abiotic complexation of DRP. The BP helps to maintain the Ca and DRP concentration in soil porewater. Both the soil and the BP layer function to remove elevated levels of DRP from the water column. The removal rates generally fit first-order reaction kinetics with rapid initial uptake which decreases with time. Biological uptake dominates the uptake mechanisms of BP. The P uptake capacity becomes saturated upon continued loading. A condition reached more rapidly for bare soils than when the BP layer is present. The loss of the BP layer would result in decreased DRP removal efficiency and a flux of P and Ca from the soil.

CHAPTER 5
ABIOTIC TRANSFORMATIONS OF INORGANIC PHOSPHORUS IN THE WATER COLUMN

Introduction

The bioavailability of P in aquatic environments is regulated by biotic (see Chapters 3 and 4) and abiotic processes. Abiotic processes, such as adsorption and precipitation, are a function of solubility related factors which include pH, ion activities, temperature, and the presence of interferring substances.

Calcite ($CaCO_3$) precipitation is often observed in hardwater systems during periods of high photosynthetic activity (Murphy et al., 1983). In the pH range of most natural hardwater systems, (pH = 7 - 10) HCO_3^- is the dominant carbonate ion (Butler, 1982). In the simplest form, photosynthesis affects calcite formation via the reaction

$$Ca^{2+} + 2HCO_3^- \leftrightarrow CO_2 + H_2O + CaCO_3 \quad [5\text{-}1]$$

by reducing the carbon dioxide and shifting the reaction to the right (Gleason, 1972; Paasche and Brubak, 1994). In addition to CO_2 numerous species of algae have the ability to utilize HCO_3^- in photosynthsis (Paasche and Brubak, 1994; McConnaughey, 1991). Calcification accompanies the photosynthetic utilization of HCO_3^-. Calcification

$$Ca^{2+} + CO_2 + H_2O \leftrightarrow CaCO_3 + 2H^+ \quad [5\text{-}2]$$

generates excess protons which are then used to manufacture twice the CO_2 as was consumed during calcification via

$$2HCO_3^- + 2H^+ \leftrightarrow 2CO_2 + 2H_2O \quad [5\text{-}3]$$

which provides the net reaction given in equation [5-1] (McConnaughey, 1991). Under high photosynthetic activity, where free CO_2 is limited short supply, the ability to use

HCO_3^- to maintain photosynthesis provides an advantage over organisms without this ability (King, 1970).

A concurrent reduction in water P concentrations during $CaCO_3$ formation has been reported in hardwater lakes (Murphy et al., 1983; Murphy and Prepas, 1990;), in biological wastewater treatment systems (Moutin et al., 1992; Arvin, 1983; Ferguson et al., 1970), and under laboratory conditions (Ferguson et al., 1973; House et al., 1986; Kleiner, 1990; Otsuki and Wetzel, 1972; Diaz et al., 1994). The reduction in solution P concentration was due to coprecipitation of ortho-P with the precipitating $CaCO_3$. Otsuki and Wetzel (1972) suggest that coprecipitation can occur either by surface adsorption or by the formation of mixed crystals during crystal growth. The form of coprecipitation depends on the rate of precipitation, agglomeration, and miscibility of the compounds (Otsuki and Wetzel, 1972). Among the conditions that encourage coprecipitation are relatively high Ca^{2+} concentrations, high pH (> 7.5), low concentrations of competing ions, and the presence of seed crystals (Jenkins et al., 1971; Diaz et al., 1994; Kleiner, 1990; Moutin et al., 1992; House et al., 1986; Arvin, 1983).

Conditions in oligotrophic areas of the northern Everglades Water Conservation Areas (WCA) should facilitate the coprecipitation of P with $CaCO_3$. The interior of WCA-2A is characterized as having highly mineralized, alkaline conditions, generally shallow water depths, and high photosynthetic activity, derived largely from the calcareous periphyton which covers much of the area (Koch and Reddy, 1992; Rader and Richardson, 1992). The periphyton of WCA-2A is composed largely of blue-green algae (cyanobacteria), such as *Schizothrix calcicola* (Ag.) Gom. and *Scytonema hofmannii* Ag., which are known to produce calcareous encrustations (Gleason, 1972). Cyanobacterial periphyton, collected from an oligotrophic area of the Everglades, contained approximately 50% dry weight as $CaCO_3$ (see Chapter 2). It is hypothesized that P coprecipitates with $CaCO_3$ in the water column, on the periphyton surfaces, or in the soil and becomes part of the long-term mineral P storage. A linear relationship between long-term P storage and Ca accumulation has been shown for Everglades soils (Reddy et al. 1993).

Hydroxyapatite [$Ca_5(PO_4)_3(OH)$, HAP] is the most thermodynamically stable Ca-P mineral suspected of forming in alkaline water systems. The generally accepted model of HAP formation is that P initially adsorbs to the surfaces of $CaCO_3$ and then through rearrangement produces phosphate heteronuclei that eventually become hydroxyapatite (Murphy and Prepas, 1990; Griffin and Jurniak, 1974). Different authors disagree as to the mineral phase of Ca-P that forms during coprecipitation. Several Ca-P minerals are metastable to HAP including , β-tricalcium phosphate [β-$Ca_3(PO_4)_2$, B-TCP], octacalcium phosphate [$Ca_4H(PO_4)_3 \bullet 2.5\ H_2O$, OCP], dicalcium phosphate [$CaHPO_4$, DCP aka. monetite] and dicalcium phosphate dihydrate [$CaHPO_4 \bullet 2\ H_2O$, DCPD aka. brushite] (Moutin et al., 1992). The form of Ca-P that precipitates is largely determined by the concentration of Ca, P, and the pH (Moutin et al., 1992; Ferguson et al., 1970; Griffin and Jurniak, 1974)

Solubility measurements provide a method for identifying which Ca-P minerals are suspected of being stable and in equilibrium with environmental conditions. Geochemical modeling provides a means for predicting possible solid phases. Equilibrium stability of a Ca-P mineral is thermodynamically favored if the ion activity product (IAP), calculated for a given set of conditions, is greater than the equilibrium constant (K) for that mineral. Conversely, if $K > IAP$, the mineral is not stable and would not form under those conditions. A number of chemical speciation models have been used to predict the theorectically stable solid Ca-P phases in aquatic systems including: SOILCHEM (Sposito and Coves, 1988; Diaz et al., 1994; Olila and Reddy, 1995), GEOCHEM (Moore et al., 1991), and WATEQ (or variants) (Murphy and Prepas; 1990; House et al, 1986).

Objectives of this study were to: determine the influence of physicochemical changes (pH, DO, etc.) of the water column on complexation/precipitation reactions between Ca and P, trace precipitation and solubilization through a range of physicochemical changes, and identify the form of probable in situ P containing mineral species so that predictions can be made about their stability.

Materials and Methods

Site Description

Surface water, benthic periphyton (BP), surficial soils, intact cores and interstitial water samples were collected from a site designated 217 in the interior of Water Conservation Area 2A (WCA-2A-217) (26° 17.17' N, 80° 24.73' W ± 30 m) of the Florida Evergaldes. The hydrology of WCA-2A is managed to provide water supply during the dry season and water storage during the rainy season. Nutrient laden water, resulting from drainage of the Everglades Agricultural Area (EAA), is routinely pumped into WCA-2A across its northern border. Steady external nutrient loading has resulted in soil and water column P concentration gradient, with higher P concentrations near inflow structures and background P contents at interior sites (SWIM, 1992; Koch and Reddy, 1992: DeBusk et al., 1994). The unimpacted interior of WCA-2A is characterized by peat soils, patchy sawgrass stands (*Cladium jamaicense* Crantz.), and abundant periphyton growth. The periphyton at WCA-2A-217 is dominated by calcareous blue-green algae (cyanobacteria) such as *Schizothrix calcicola* (Ag.) Gom. and *Scytonema hofmannii* Ag., as well as hardwater diatoms (Swift and Nicholas, 1987). This calcareous periphyton exists as as a thick carpet covering the soil surface, as coatings on the submerged portions of dead sawgrass stems, and as mats floating on the water surface.

Phosphorus Precipitation: Physicochemical Conditions

A reactor system was used to to determine the affects water column physicochemical changes have on Ca-P precipitation reactions. Periphytic removal of P from the water column was dominated by biotic uptake. Generally < 10% of the P was removed by abiotic processes (see Chapters 3 and 4). Water-only reactor experiments were conducted to avoid competition of biotic uptake which would occur if periphyton was present.

Laboratory reactors

Floodwater was collected from WCA-2A-217, brought to the laboratory, and 0.2 µm filtered (Gelman Supor, Gelman, Inc.). Subsamples were collected for chemical characterization (n = 3). Measured volumes of 1.8 L of water were dispensed into nine 2 L reaction vessels. Each reaction vessel was equipped with a pH electrode and gas inlet and outlet tubes. The pH of water in each vessel was continuously monitored. The pH electrodes were connected to a potentiometric multiplexer (Diamond General Corp., Ann Arbor, MI) which was then connected to a 12V DC pH meter. Output from the pH meter was connected to a datalogger (CR10, Campbell Scientific Inc., Logan UT). The datalogger was programmed to switch the multiplexer through the electrodes at 10 s intervals. Electrode output was recorded to internal datalogger memory then averaged over a ten minute interval and output to permanent storage. This resutled in 10 min average pH values based on 6 or 7 interim readings for each vessel. Reaction vessels were incubated in triplicate under constant stirring on magnetic stirplates. Temperature in the reaction vessels averaged 27 ± 4° C as measured by alcohol thermometers inserted in several vessels.

Three treatments were imposed on triplicate reactors. Water in Treatment I recieved a constant stream of CO_2-free air at flowrates of approximately 13 ml min^{-1} as measured by flowmeters (Manostat Calcuflow Flowmeter 36-541-035, New York, NY)

The CO_2-free air was obtained by scrubbing compressed air through a 10 *M* NaOH trap followed by a water-filled trap to rehydrate the gas. Treatment II consisted of triplicate reaction vessels receiving a continuous stream of CO_2-enriched air (3% CO_2) at flowrates of about 2 ml min^{-1}. Treatment III received alternating CO_2-free air (13 ml min-1) and CO_2-enriched air (2 ml min-1) on 12 h intervals. The alternating addition or removal of CO_2 in the third treatment was meant to represent the production and consumption, respectively, of CO_2 under periphytic photosynthesis and respiration. Switching of gasses in Treatment III was accomplished by electrically operated, timer controlled solenoids.

Experimentation was initiated after obtaining a steady pH, or steady pH cycle. The vessels were allowed to equilibrate for six days after which all vessels were spiked with KH_2PO_4 to bring the P concentration to a nominal 100 $\mu g\ L^{-1}$. Water samples (10 ml), collected every 12 h after spiking, were 0.2 µm filtered and analyzed for dissolved reactive P (DRP). On day 8 (48 h after spiking), water samples were collected from the reaction vessels of Treatment III (alternating pH) at 0, 0.25, 0.5, 2, 3, 4, 6, 8, 10,and 12 h after CO_2-enriched air was introduced. Similarly, samples were collected at the same intervals after the introduction of CO_2-free air (day 8.5). The reaction vessels of the other two treatments continued to be sampled at 12 h intervals. Occasionally, larger samples (60 ml) were collected and split into filtered and unfiltered subsamples. Unfiltered samples were analyzed for pH, alkalinity, electrical conductivity (EC), total C (TC), total inorganic C (TIC), total organic C (TOC), and total P (TP). Filtered samples were analyzed for DRP, total dissolved C (TDC), dissolved organic C (DOC), dissolve inorganic C (DIC), and selected ions. Added P brought the P concentration to approximately 100 $\mu g\ L^{-1}$ in the vessels of Treatments I and II. However, the P concentration in Treatment III, the alternating pH treatment, was unintentionally spiked to approximately 90, 110, and 140 $\mu g\ L^{-1}$. This variation in spiking led to higher than expected variation in the treatment means. Therefore, at 13.5 days the reaction vessels of Treatment III were again spiked: to normalize P concentrations in triplicate reactors, and to increase the activity of P in solution for easier determination of treatment effects. Samples were collected from treatment III reactors at 0, 4, 8, 12, 16, 20, 24, 28, 32, 36 h after the second spiking.

To determine if a reduction in pH from approximately 9.0 to near neutrality would cause a corresponding resolubilization of complexed P, the gas into Treatment I (CO_2-free air) was changed to CO_2-enriched air at day 13.5. Similarly, reaction vessels of Treatment II, previously receiving CO_2-enriched air, with an approximate pH = 7.0, was changed to CO_2-free air. Treatment I had elevated pH at the time of P-spiking. Changing the gas flows to Treatment II allowed determination of the rate and extent of P complexing with increasing pH. Samples were collected at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 11, 15, 19, and 24 h after switching gasses. Treatment I was terminated on the 17th day , after a substantial portion of P was returned to solution. Treatments II and III were terminated on day 26.

Chemical Characteristics of Water, BP Interstitial Water, and Soil Porewater

Reactor studies supplied a set of artificailly controlled conditions differing from the physicochemical conditions of WCA-2A. Surface water, and the interstitial water of the BP and soil from WCA-2A-217 was evaluated to determine if field conditions were conducive to the formation and stability of Ca-P minerals.

Porewater equilibrators

Profiles of soluble constituents in soil porewater, the BP interstitial water, and in the water column were sampled with porewater equilibrators. The equilibrators were constructed of Plexiglas according to the methods of Hesslein (1976). Equilibrators had recesses of approximately 8 cm^3 volume spaced at 1 cm intervals. The cells of the equilibrators were initially filled with distilled, deionized water that had been deaerated by purging with N_2 gas. Cells were covered with a 0.2 μm Nucleopore polycarbonate membrane (Nucleopore Corp., Pleasanton, CA) which was covered by a protective layer of 1 µm Spectra mesh nylon (Spectrum Corp., Los Angeles, CA). Once assembled, the equilibrators were placed into air-tight rectangular carrying cases filled with distilled, deionized, deaerated water. The equilibrator containing carrying cases were then purged with N_2 gas for about 16 h before being sealed with air-tight lids and transported to the field site.

Enclosures had been inserted into the peat soils of WCA-2A-217 14 days before the equilibrators were installed (see Chapter 4). Enclosures, made from Lexan sheeting, had been installed to isolate microcosm sections of the peat wetland. Eight equilibrators were installed one in each of eight enclosures. Four of the enclosures had intact BP layers while the other four had BP removed. Benthic periphyton was removed from the periphyton-less enclosures at the time of their installation. Enclosures had fine mesh openings to restrict the immigration of BP while allowing exchange of water. Equilibrators were inserted into the peat soils so that at least 15 cells (15 cm depth) were below the soil/water interface. Cells extended through the BP layer, if present, and into the overlying floodwater. After 15 days, the porewater equilibrators were removed. The soil/water, soil/BP, and BP/water interfaces were marked at the time of retrieval. Water was immediately removed from each cell with a hypodermic syringe. Syringes were inserted into rubber stoppers, placed into an icewater bath, and transported to the laboratory.

Samples were analyzed individually (1 cm increments) for pH and DRP or were combined in 5 cm increments for analysis of TDC, DIC, DOC, and selected ions. Values resulting from 1 cm increments were averaged over 5 cm depths. All cells containing BP interstitial water were combined or averaged to provide 1 BP sample for each equilibrator. Depth of BP layers in the four BP-containing replicate enclosures averaged 8.00 ± 0.35 cm (mean ± SE). Resultant average values presented are mean ± SE for four replicate equilibrators regardless of combining.

Oxygen and pH Profiles of Soil Columns with and without BP

Porewater equilibrators provide water physicochemical information on a scale of one to several cm. To obtain changes in the pH and DO conditions with depth in the water-periphyton-soil column on mm scale, electrodes were driven through intact cores. Profiles in pH and dissolved oxygen (DO) were conducted using cores collected from the field site on August 20, 1994. These cores contained 20 cm of water covering a 4 cm layer of BP and 20 cm of peat soil. The BP layer was gently removed from one core by suction. After three days equilibration under a light booth, providing 10 W m^{-2} on a 16 h light - 8 h dark cycle, micro-pH and DO electrodes (Diamond General Corp., Ann Arbor, MI) were simultaneously driven into the cores by a mechanical drive unit at 10 cm h^{-1}. Measurements of pH and DO along the depth profile were madw every 10 s.

Analytical Methods

Unfiltered water samples were characterized for pH, alkalinity (Method 2320, APHA, 1992), and EC (YSI Model 31, Yellow Springs Instruments, Yellow Springs, OH) (Method 2510, APHA, 1992) immediately after collection. Unfiltered samples were analyzed for TC, TIC, and TOC (on a Rosemont Analytical Inc. DC-190 High Temperature TOC Analyzer, Dohrman Division, Santa Clara CA) and for TP by colorimetric analysis following persulfate oxidation (Method 4500-P-B, APHA, 1992). Filtered (0.2 µm) (Cameo acetate membrane, MSI Inc.) water samples from the reaction vessels and the water from porewater equilibrators were collected and generally were analyzed within 24 h by colorimetric analysis of DRP (Method 4500-P-F, APHA, 1992) on an AutoAnalyzer II (Technicon Industrial Systems, Tarrytown, NY). Porewater equilibrator samples were placed immediately into ice water upon collection in the field. Occasionally, filtered reactor samples were frozen immediately after collection and stored until analyzed. Filtered water samples were also collected and analyzed for TDC, DIC and DOC (as above for total C forms), SO_4^{2-} (by ion chromatography using a Dionex 4500i, Dionex Corp. Sunnyvale, CA) (Method 4110, APHA, 1992), and selected cations; Ca, Mg, K, Na, Cl, Si, Zn, Cu, Mn, Al, and Fe by Inductively Coupled Argon Plasma Spectrometry (ICP) (Method 3120B, APHA, 1992).

Mineral Equilibria Calculations

Results of periodic sampling of the reaction vessels and from water data obtained by analysis of porewater equilibrator samples were used to develop separate data sets for geochemical modeling. Chemical speciation of ions in the water phase were determined from ion activities as calculated by the computer program SOILCHEM (Sposito and Coves, 1991). Input consisted of measured concentrations of P (as DRP), Ca, Mg, K, Na, Cl, Si, and SO_4^{2-}, pH and estimates of CO_3^{2-} (as DIC) and ionic strength (estimated from EC measurements). Ionic strength was calculated by the program. Precipitation of solids was not assumed to occur in calculating the ion activities. SOILCHEM estimates of the Ca^{2+}, PO_4^{3-}, Mg^{2+}, and HCO_3^- activities were corrected for ion pairs and complex ions such as $CaCO_3$, $CaHCO_3^+$, $CaSO_4$, $CaHPO_4$, $CaPO_4^{2-}$, and others (Olila and Reddy,1995). Solubility diagrams were created using the SOILCHEM-generated ion activities with equilibrium constants (log K) obtained from Lindsay (1979). Solid phases considered in the diagrams include hydroxyapatite [$Ca_5(PO_4)_3(OH)$, HAP], β-tricalcium phosphate [β-$Ca_3(PO_4)_2$, B-TCP], octacalcium phosphate [$Ca_4H(PO_4)_3 \cdot 2.5\ H_2O$, OCP], dicalcium phosphate [$CaHPO_4$, DCP aka. monetite] and dicalcium phosphate dihydrate [$CaHPO_4 \cdot 2\ H_2O$, DCPD aka. brushite].

X-ray Diffraction of BP and Surficial Soils

Benthic periphyton, and soils from the surface 0 - 2 cm, and 2 - 5 cm depths were collected in intact cores from the field site in on April 10, 1995 (see Chapter 2). Representative samples were air-dried and ground (#100 mesh) and analyzed by X-ray diffraction. Samples were prepared as powder mounts and analyzed on a computer controlled x-ray diffraction system with stepping motor and a graphite crystal monochrometer. Copper K-α radiation was used at a scan rate of 2 degrees 2θ min^{-1}.

Results and Discussion

Phosphorus Solubility as Influenced by Fluctuating pH and PCO_2

The DRP concentration of WCA-2A-217 water accounted for 50-60% of water TP (Table 5-1). Initial pH of the water was 8.1. Alkalinities (mg $CaCO_3$ L^{-1}) were similar to those reported in Swift and Nicholas (1987) (245 mg L^{-1}), and in Chapter 2 (this document) (230 mg L^{-1}).

The pH of Treatment I (CO_2-free air for 13.5 days followed by CO_2-enriched air) water was maintained above pH = 8.6 for several days before spiking at 100 μg L^{-1} occurred (Fig. 5-1). The DRP concentration in decreased by approximately 60% within 7.5 days of spiking (Fig. 5-2). Low mean DRP concentration for this treatment occurred on Day 13.5 and was 44 μg L^{-1} (Table 5-2 and Fig. 5-2). A sharp decrease in pH occurred when water in these vessels received CO_2-enriched air (Fig. 5-1). Samples collected at short time intervals during this period of changing pH showed about two-thirds of the previously removed P was returned to solution within 12h. At the conclusion of this treatment (17.9 days), approximately 20% of the added P was removed from solution.

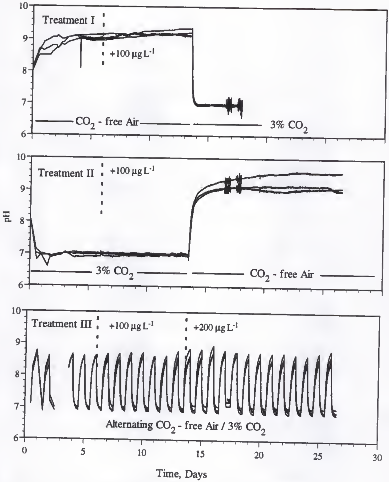

Fig. 5-1. Solution pH in reaction vessels, by treatment, under varying CO_2.

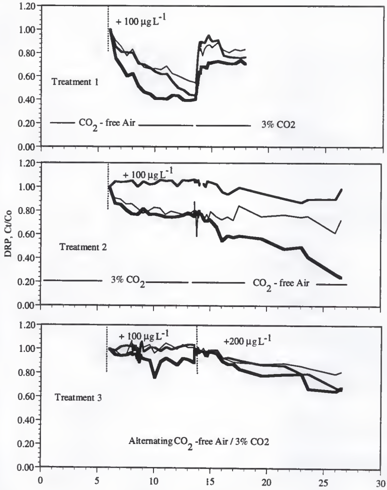

Fig. 5-2. Ratio of reaction vessel dissolved reactive phosphorus (DRP) as a proportion of initial DRP spiked, vs. time. Initial spiking occurred at the end of the sixth day of gas treatments.

Table 5-1. Initial chemical characterization of WCA-2A-217 water used in the reaction vessel study (Mean ± SE, n=3).

Bulk H_2O

Parameter	Units	
DRP	$\mu g\ L^{-1}$	7.3 ± 0.5
TP	$\mu g\ L^{-1}$	13.9 ± 3.7
pH		8.13 ± 0.02
EC	$mS\ m^{-1}$	101.0 ± 0.0
Total Alkalinity	$mg\ CaCO_3\ L^{-1}$	292 ± 1
Carbonate Alk.	$mg\ CaCO_3\ L^{-1}$	0 ± 0
TC	$mg\ L^{-1}$	87.8 ± 3.9
TIC	$mg\ L^{-1}$	43.4 ± 1.1
TOC	$mg\ L^{-1}$	44.4 ± 3.1
TDC	$mg\ L^{-1}$	97.3 ± 4.2
DIC	$mg\ L^{-1}$	46.6 ± 2.5
DOC	$mg\ L^{-1}$	50.7 ± 2.0
Ca	$mg\ L^{-1}$	60.3 ± 2.9
Mg	$mg\ L^{-1}$	32.7 ± 0.5
K	$mg\ L^{-1}$	9.5 ± 0.5
Na	$mg\ L^{-1}$	149 ± 9
Cl	$mg\ L^{-1}$	198 ± 14
Si	$mg\ L^{-1}$	6.5 ± 0.2
SO_4	$mg\ L^{-1}$	14.5 ± 0.7

Zn, Cu, Mn, Al, and Fe all < 0.1 $mg\ L^{-1}$.

Calcite precipitated 2.5 days after CO_2-free air was introduced into the water. Evidence for $CaCO_3$ precipitation was a "cloudy" appearence in the water, an approximately one-third reduction in the Ca ion concentration of the solution, and an increase in the carbonate alkalinity (Tables 5-1 and 5-2). Solid $CaCO_3$ was therefore present at the time of spiking which suggests that DRP removed from solution would have occurred via adsorption to solid $CaCO_3$. Expanding reaction [5-1], CO2-free air purging removed free CO_2 from solution causing a shift to the right in the carbonate equilibrium

$$2HCO_3^- \leftrightarrow CO_2 + CO_3^{2-} + H_2O \qquad [5\text{-}5]$$

where the bicarbonate is derived from alkalinity. This increases the activity of CO_3^{2-} in solution which was then available for precipitation with Ca^{2+} ions (King, 1970).

The disappearance of DRP from solution follwed first-order relationship

$$C = Co\ e^{-kt} \qquad [5\text{-}6]$$

where C is the DRP concentration at any time, Co is the initial DRP concentration, k is the first order rate constant, and t is time in days. This resulted in the expression $C = 81.5\ \mu g\ L^{-1}\ e^{-0.087\ t}$ to describe the DRP depletion. The first order rate constant (k) (-0.087) has the units day^{-1}. This expression fit the data with an $r^2 = 0.93$ and underestimated Co by 14 $\mu g\ L^{-1}$. The presence of precipitated $CaCO_3$(cloudiness) persisted for over 12 h after gas flow was switched to CO_2-enriched air, and within 24 h the solutions became clear. Evidently the reaction shifted back to the left, with the CO_2 addition, thus consuming CO_3^{2-}. This was further supported by very little or no change in total alkalinity, but there was an appreciable loss of carbonate alkalinity. Diaz et al. (1994) observed that the total alkalinity was not affected in water receiving gas flows with CO_2 concentrations varying between 0 and 3%.

The DRP concentration of the water in Treatment II (CO_2-enriched air for 13.5 days followed by CO_2-free air) decreased by 12% in 7 days following spiking (Table 5-3 and Fig. 5-2). The pH during this period was near neutral (Fig. 5-1). The behavior of

Table. 5-2. Chemical characteristics of reaction vessel water from Treatment I (Mean ± SE, n=3).

Treatment I		CO_2-free Air			3% CO_2
Parameter	Units	Spike Day 6	Day 8	Day 13.5	Day 17.9
DRP	µg L^{-1}	95.4 ± 5.0	71.2 ± 3.0	43.8 ± 2.0	73.0 ± 0.7
TP	µg L^{-1}	105.4 ± 5.2	91.9 ± 13.9	102.6 ± 7.3	109.3 ± 5.4
pH		8.62 ± 0.02	8.72 ± 0.02	8.72 ± 0.09	6.95 ± 0.00
EC	mS m^{-1}	95.3 ± 9.3	103.3 ± 12.3	107.3 ± 13.9	122.3 ± 14.7
Total Alkalinity	mg $CaCO_3$ L^{-1}	274 ± 2	255 ± 4	232 ± 10	255 ± 2
Carbonate Alk.	mg $CaCO_3$ L^{-1}	45 ± 4	47 ± 3	41 ± 8	0 ± 0
TC	mg L^{-1}	84.7 ± 1.8	83.4 ± 1.5	77.3 ± 1.0	98.7 ± 0.6
TIC	mg L^{-1}	37.0 ± 0.8	34.2 ± 0.8	30.5 ± 0.8	51.6 ± 0.4
TOC	mg L^{-1}	47.7 ± 1.3	49.2 ± 0.7	46.8 ± 0.5	47.0 ± 0.6
TDC	mg L^{-1}	86.1 ± 0.8	82.8 ± 3.3	82.7 ± 1.0	99.3 ± 2.0
DIC	mg L^{-1}	35.5 ± 1.7	36.7 ± 1.0	34.3 ± 0.7	52.9 ± 0.2
DOC	mg L^{-1}	50.6 ± 1.0	46.0 ± 2.3	48.4 ± 0.7	46.4 ± 1.8
Ca	mg L^{-1}	42.8 ± 2.4	43.3 ± 1.4	40.6 ± 1.6	67.9 ± 0.5
Mg	mg L^{-1}	31.9 ± 0.7	31.5 ± 2.0	32.0 ± 0.2	32.4 ± 0.0
K	mg L^{-1}	59.6 ± 38.3	71.9 ± 48.5	78.8 ± 49.8	81.9 ± 49.0
Na	mg L^{-1}	146 ± 3	145 ± 7	149 ± 1	147 ± 1
Cl	mg L^{-1}	240 ± 35	256 ± 53	274 ± 48	274 ± 48
Si	mg L^{-1}	6.6 ± .2	6.8 ± 0.4	6.7 ± 0.1	7.3 ± 0.2
SO_4	mg L^{-1}	13.1 ± 0.3	13.0 ± 0.7	13.1 ± 0.1	13.0 ± 0.1

Zn, Cu, Mn, Al, and Fe all < 0.1 mg L^{-1}.

Table. 5-3. Chemical characteristics of reaction vessel water from Treatment II (Mean ± SE, n=3).

Treatment II		3% CO_2			CO_2-free Air
Parameter	Units	Spike Day 6	Day 8	Day 13.5	Day 26.5
DRP	$\mu g\ L^{-1}$	101.7 ± 4.2	90.5 ± 6.0	88.4 ± 7.7	64.5 ± 20.2
TP	$\mu g\ L^{-1}$	122.9 ± 6.8	119.0 ± 5.1	102.7 ± 5.7	105.5 ± 15.1
pH		7.16 ± 0.11	7.09 ± 0.04	7.27 ± 0.06	8.90 ± 0.1
EC	$mS\ m^{-1}$	84.3 ± 2.2	101.5 ± 3.3	112.0 ± 6.4	112.7 ± 8.7
Total Alkalinity	mg $CaCO_3\ L^{-1}$	278 ± 2	281 ± 3	272 ± 4	252 ± 8
Carbonate Alk.	mg $CaCO_3\ L^{-1}$	0 ± 0	0 ± 0	0 ± 0	106 ± 16
TC	$mg\ L^{-1}$	98.3 ± 0.6	95.8 ± 3.4	99.7 ± 1.0	102.8 ± 2.0
TIC	$mg\ L^{-1}$	52.1 ± 0.5	49.3 ± 2.3	51.5 ± 0.6	46.8 ± 2.2
TOC	$mg\ L^{-1}$	46.2 ± 0.8	46.5 ± 1.3	48.2 ± 0.7	55.9 ± 0.7
TDC	$mg\ L^{-1}$	102.7 ± 3.4	95.3 ± 5.0	105.0 ± 3.0	97.2 ± 2.2
DIC	$mg\ L^{-1}$	49.9 ± 1.9	44.7 ± 4.1	53.6 ± 0.7	45.0 ± 1.5
DOC	$mg\ L^{-1}$	52.8 ± 1.9	50.5 ± 1.0	51.5 ± 3.4	52.3 ± 2.8
Ca	$mg\ L^{-1}$	64.1 ± 0.9	58.1 ± 4.7	65.9 ± 0.3	61.0 ± 5.9
Mg	$mg\ L^{-1}$	33.7 ± 0.5	33.2 ± 0.4	32.9 ± 0.1	33.6 ± 0.5
K	$mg\ L^{-1}$	30.5 ± 9.1	35.4 ± 9.8	61.7 ± 23.4	84.9 ± 30.2
Na	$mg\ L^{-1}$	154 ± 0	152 ± 1	152 ± 1.2	138 ± 3
Cl	$mg\ L^{-1}$	214 ± 6	223 ± 12	252 ± 21	267 ± 29
Si	$mg\ L^{-1}$	6.3 ± 0.1	6.5 ± 0.2	6.4 ± .2	8.0 ± 0.4
SO_4	$mg\ L^{-1}$	14.5 ± 0.5	11.9 ± 0.7	13.3 ± 0.1	13.5 ± 0.1

Zn, Cu, Mn, Al, and Fe all < 0.1 $mg\ L^{-1}$.

individual vessels varied greatly. Actual reductions in DRP of the separate replicates during this period ranged from 0 to 25%. Two of the reaction vessels had DRP reductions > 20% but the third vessel showed a slight increase in DRP skewing the results (Fig. 5-2). The pH increased rapidly when the vessels began receiving CO_2-free air (Fig. 5-1). Average DRP depletion during this period of high pH was about 37% of the initial spike. Again, the DRP results were highly variable between replicates with DRP depletion ranging 1 - 76% (Fig. 5-2). Only one of the three replicate vessels became cloudy during the period of high pH. This replicate also had the greatest (76%) reduction in DRP concentration. In this replicate approximately one-third of the DRP reduction occurred under CO_2 enrichment with pH = 7.0. The remaining reduction ocurring during the CO_2-free air stage. At the conclusion of this study the solution from this vessel was 0.2 μm filtered, the precipitate collected, and analyzed by X-ray diffraction. The precipitate was shown to be $CaCO_3$.

Treatment III (12 h CO_2-free air / 12 h CO_2-enriched air) had solution pH that fluctuated approximately 1.8 pH units every 12h (Fig. 5-1). There was virtually no DRP reduction in the 7days following the first spike (Fig. 5-2). Increasing the DRP concentration by an additional spiking to a DRP concentration nominally at 200 $\mu g\ L^{-1}$, did not encourage precipitation (Fig. 5-2). Total DRP reduction averaged 28% of added DRP by the end of the study.

Gleason and Spackman (1974) also observed diurnal shifts in solution Ca^{2+} concentration and pH associated with photosynthesis and respiration of the algal mat in the northern Everglades. Their observed Ca^{2+} shifts were similar to those reported here when comparing Ca concentrations between CO_2-free and CO_2-enriched air. No shift in solution Ca concentrations were observed in Treatment (III), which was supposed to mimic diurnal pH/PCO2 fluctuations. The pH varied diurnally from 7.6 - 8.2 in the Gleason and Spackman study whereas Treatment III pH varied over a greater range (7.0 -

8.8). The higher pH attained in this study should have encouraged $CaCO_3$ precipitation and DRP coprecipitation.

The water used in the reaction vessels was filtered through 0.2 µm filters to remove living cells and other suspended particles. Filtering, and covering the vessels with aluminum foil was done in an attempt to diminish biotic P uptake. In preliminary studies autoclaving WCA-2A water caused increases in pH and precipitation (cloudiness) to occur, presumably because degassing at high temperature caused loss of CO_2. It was concluded that autoclaving was too severe a sterilization method. Unfortunately, 0.2 µm filtering might not have entirely eliminated biotic activity. By the end of the study reactors of Treatment II and III had formed a visible, light, growth of periphytic material at the ends of the pH electrodes. This growth was not visible until the last few days of the experiment. There was no visible growth in the reactors of treatment I due to shorter duration. Additional evidence of biological growth towards the end of the study is a slight increase in water TOC concentrations (Table 5-1, 5-3, 5-4). Total organic carbon remained constant in Treatment I (Table 5-2), for at least the first 13.5 days of Treatment II (Table 5-3), and for at least the first 8.5 days of Treatment III (Table 5-4). The TOC values at the end of the study in Treatment II and III were 19% and 9% greater, respectively, than the average TOC concentrations from previous measurements.

The concentration of K and Cl was highly variable among reaction vessels and between treatments but there was a general increase in concentration of these ions with time. This is a consequence of ionic diffusion from permanently installed liquid-filled pH electrodes. In Treatments I and II, which showed large changes in K and Cl with time, the EC also increased with time (Table 5-2 and 5-3). Electrical conductivity, K and Cl concentration increased only slightly in Treatment III (Table 5-4).

Table. 5-4. Chemical characteristics of reaction vessel water from treatment III (Mean ± SE, n=3).

Treatment III		Alternating (12 h) cycles of CO_2-free Air and 3% CO_2			
Parameter	Units	Spike 1 Day 6	Day 8	Day 8.5	Day 26.5
DRP	µg L^{-1}	115.9 ± 17.6	117.6 ± 15.8	112.3 ± 15.1	140.8 ± 9.6
TP	µg L^{-1}	151.3 ± 22.6	149.9 ± 15.5	105.9 ± 16.1	159.6 ± 8.8
pH		8.12 ± 0.03	8.22 ± 0.07	7.02 ± 0.03	7.38 ± 0.18
EC	mS m^{-1}	85.0 ± 0	94.7 ± 1.8	93.7 ± 1.3	99.3 ± 0.7
Total Alkalinity	mg $CaCO_3$ L^{-1}	288 ± 1	286 ± 2	286 ± 2	270 ± 6
Carbonate Alk.	mg $CaCO_3$ L^{-1}	0 ± 0	8 ± 8	0 ± 0	0 ± 0
TC	mg L^{-1}	93.8 ± 1.8	92.4 ± 1.3	97.9 ± 1.2	114.2 ± 1.7
TIC	mg L^{-1}	45.7 ± 1.1	44.9 ± 1.2	52.4 ± 0.6	63.0 ± 0.9
TOC	mg L^{-1}	48.1 ± 0.7	47.5 ± 1.1	45.5 ± 0.6	51.2 ± 2.3
TDC	mg L^{-1}	97.9 ± 2.7	103.3 ± 0.8	97.3 ± 2.5	117.1 ± 0.9
DIC	mg L^{-1}	46.4 ± 0.1	49.4 ± 1.5	48.0 ± 1.6	61.6 ± 0.7
DOC	mg L^{-1}	51.5 ± 2.7	53.8 ± 0.7	49.3 ± 3.2	55.5 ± 1.6
Ca	mg L^{-1}	61.8 ± 2.0	65.1 ± 1.0	61.5 ± 2.2	67.7 ± 0.6
Mg	mg L^{-1}	32.7 ± 1.1	33.9 ± 0.2	31.7 ± 0.5	33.7 ± 0.2
K	mg L^{-1}	11.5 ± 0.4	12.0 ± 0.5	11.6 ± 0.5	16.2 ± 1.9
Na	mg L^{-1}	151 ± 5	155 ± 1	146 ± 1	138 ± 1
Cl	mg L^{-1}	198 ± 5	204 ± 2	198 ± 2	202 ± 0
Si	mg L^{-1}	6.5 ± .3	7.1 ± 0.1	6.7 ± 0.2	7.9 ± 0.1
SO_4	mg L^{-1}	13.4 ± 0.5	13.8 ± 0.1	12.9 ± 0.3	13.2 ± 0.1

Zn, Cu, Mn, Al, and Fe all < 0.1 mg L^{-1}.

Chemical Characteristics of Water, BP Interstitial Water, and Soil Porewater

Detailed comparisons of the chemical composition of porewater from the equilibrators were previously presented (see Chapter 4). Chemical characteristics of porewaters at selected positions in the water-BP-soil column are repeated here for reference (Table 5-5).

The DRP measured from porewater equilibrator cells is assumed to be in equilibrium with that adsorbed to the solid phase (Hesslein, 1975; Carignan, 1983). The DRP concentration was higher in the porewater under BP than in the -BP treatment and was probably due to the lower pH. The pH was greatest in the floodwater (pH = 7.8) and decreased to about pH = 7.0 within 15 cm soil depth (Table 5-5). The pH of the BP layer was the same as that of the surficial soil layer (0-5 cm) of the -BP treatment. There is a reduction in porewater pH following the surficial layer whether that layer is BP or surface soil. The pH of the soil under BP was maintained at pH = 7.0. Removing the BP caused greater variation in the soil pH within the surficial 10 cm.

The DIC of the water above -BP soil was higher than that of the BP containing treatment. The BP layer had a higher interstitial DIC concentration in the soil when compared to the porewater in soil not covered by BP. Similarly, the Ca concentration of the porewater in the presence of BP was higher than that of the -BP system. This suggests that the lower pH tends to increase the dissolution of $CaCO_3$ under BP layers. If the DRP is associated with the $CaCO_3$ then this would account for the increased DRP in the +BP interstitial water.

The DOC and concentrations of other analytes in both systems was similar except for Si (Table 5-5). Silica content in all layers except the floodwater was greater in the treatment containing BP than without BP and is probably a function of the presence of silica containing diatoms in the periphyton.

Table 5-5. Chemical characteristics of water column, interstitial water from the benthic periphyton layer (BP) (thickness = 8.3 ± 0.35 cm), and soil porewater at three depths below soil/water column interface. Water was collected from porewater equilibrators incubated in WCA-2A-217 field enclosures between September 18 through October 3, 1995. Enclosures contained intact BP (+BP) layers or had BP removed (-BP). (Mean ± SE, n=4 unless noted † then n=3, or ‡ then n = 2). (Zn, Cu, Mn, Al, and Fe also analyzed < 0.01 mg L^{-1}).

Parameter	+BP					-BP			
	Water	BP	Soil (depth, cm)			Water	Soil (depth, cm)		
			0 - 5	6 - 10	11 - 15		0 - 5	6 - 10	11 - 15
pH	7.8 ± 0.03	7.2 ± 0.03	7.0 ± 0.05	7.0 ± 0.03	7.0 ± 0.02	7.8 ± 0.09	7.2 ± 0.05	7.1 ± 0.06	7.0 ± 0.04
					μg L^{-1}				
DRP	5.0 ± 0.6†	9.1 ± 0.7	9.4 ± 0.8	8.5 ± 0.9	8.7 ± 0.9	5.0 ± 0.4	6.4 ± 0.3	6.6 ± 0.1	7.4 ± 0.4
					mg L^{-1}				
TDC	110 ± 2	145 ± 7	158 ± 7	162 ± 4	153 ± 6	112 ± 4	136 ± 4	141 ± 10	152 ± 3
DIC	58 ± 4	87 ± 4	108 ± 4	109 ± 4	104 ± 4	70 ± 10	84 ± 5	97 ± 7	93 ± 2
DOC	52 ± 3	58 ± 3	50 ± 7	52 ± 2	50 ± 2	51 ± 3	51 ± 4	44 ± 10	59 ± 2
Ca	79 ± 3	114 ± 3	129 ± 1	127 ± 1	124 ± 2	94 ± 7	108 ± 4	110 ± 6	117 ± 3
Mg	10.0 ± 0.1	10.5 ± 0.2	12.0 ± 0.2	12.9 ± 0.1	13.0 ± 0.2	10.3 ± 0.5	11.2 ± 0.4	11.4 ± 0.5	11.8 ± 0.4
K	11.3 ± 0.7	11.4 ± 0.4	13.1 ± 0.7	13.4 ± 0.6	13.0 ± 0.6	10.3 ± 0.3	11.1 ± 0.5	11.6 ± 0.6	10.9 ± 0.5
Na	111 ± 1	108 ± 2	122 ± 5	133 ± 6	139 ± 4	112 ± 1	119 ± 4	129 ± 4	133 ± 3
Cl	148 ± 1	162 ± 8	164 ± 8	174 ± 9	187 ± 8	148 ± 2	158 ± 5	170 ± 6	184 ± 8
Si	12.6 ± 0.3	18.8 ± 0.7	20.7 ± 0.9	19.3 ± 1.3	17.7 ± 1.3	12.1 ± 0.3	14.5 ± 0.7	15.0 ± 0.6	14.5 ± 0.4
SO_4	17.2 ± 0.4‡	7.6 ± 0.9	6.1 ± 0.7	5.8 ± 0.9	6.3 ± 2.1†	16.3 ± 0.8	9.9 ± 0.7	7.1 ± 0.7	5.1 ± 0.4

Oxygen and pH Profiles of Soil Columns with and without BP

Profiles of pH (Fig. 5-3) show an abrupt decrease starting at a soil depth of 5 mm in the -BP core. The pH decreased until approximately 20 mm then remained relatively constant at a pH circa 7.6. The pH of the +BP core decreased linearly from the BP/water interface through the measured soil depth of 40 mm. The pH decreased to a lower point (pH = 7.3) at 40 mm soil depth in the presence of BP than when the BP layer was removed. The pH trends from these profiles agree with those measured in the equilibrators that the presence of BP causes the pH of the soil to decrease slightly more than in a BP-less system. This is due to increased CO_2 as a by-product of microbial respiration in the algal mat.

The DO decreased within 10 cm of either the BP/water or soil/water interface. There is an oxygenated layer of about 8 mm in the soil when the BP was not present. The lower layers of BP consume available oxygen therefore the soil layer in these cores were deplete of DO (Fig. 5-3).

Mineral Equilibria

Laboratory experiments

Solution chemistry data from laboratory experiments (Tables 5-1 thru 5-4) were used in the computer program SOILCHEM (Sposito and Coves, 1991) to determine ion activity products (log IAP) for the identification of potential P-containing solid phases. Values of log K and mineral formation reactions were obtained from Lindsay (1979) (Table 5-6). If log IAP > log K for a given mineral then the solution is supersaturated with respect to that mineral and it would be stable if present (insoluble under those conditions). Conversely, when log K > log IAP the solution is undersaturated with respect to that mineral and precipitation will not occur.

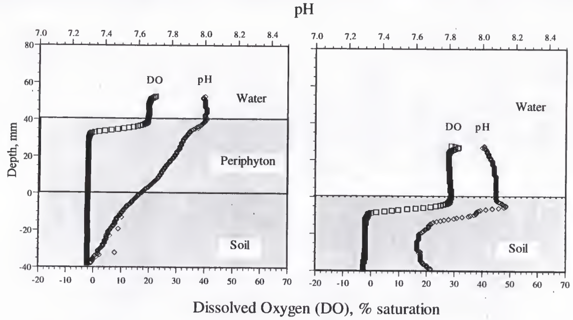

Fig. 5-3. Dissolved oxygen (DO) and pH profiles in intact cores.

Table 5-6. Equilibrium reactions and constants for selected calcium phosphates and calcium carbonates at 25° C. Reactions, and constants are taken from or calculated using Lindsay (1979).

Mineral and Equilibrium Reactions	logK
Hydroxyapatite (HAP)	
$Ca_5(PO_4)_3OH + 7H^+ \leftrightarrow 5Ca^{2+} + 3H_2PO_4^- + H_2O$	14.46
$Ca_5(PO_4)_3OH + 4H^+ \leftrightarrow 5Ca^{2+} + 3HPO_4^{2-} + H_2O$	-7.14
$Ca_5(PO_4)_3OH + 5HCO_3^- \leftrightarrow 5CaCO_3 + 3HPO_4^{2-} + H^+ + H_2O$	-16.74
β-tricalcium phosphate (B-TCP)	
$Ca_3(PO_4)_2 + 4H^+ \leftrightarrow 3Ca^{2+} + 2H_2PO_4^-$	10.18
$Ca_3(PO_4)_2 + 2H^+ \leftrightarrow 3Ca^{2+} + 2HPO_4^{2-}$	-4.22
$Ca_3(PO_4)_2 + 3HCO_3^- \leftrightarrow 3CaCO_3 + 2HPO_4^{2-} + H^+$	-9.98
Octacalcium phosphate (OCP)	
$Ca_4H(PO_4)_3 \bullet 2.5H_2O + 5H^+ \leftrightarrow 4Ca^{2+} 3H_2PO_4^- + 2.5H_2O$	11.76
$Ca_4H(PO_4)_3 \bullet 2.5H_2O + 2H^+ \leftrightarrow 4Ca^{2+} 3HPO_4^{2-} - + 2.5H_2O$	-9.84
$Ca_4H(PO_4)_3 \bullet 2.5H_2O + 4HCO_3^- \leftrightarrow 4CaCO_3 + 3HPO_4^{2-} + 2H^+ + 2.5H_2O$	-17.52
Monetite (dicalcium phosphate, DCP)	
$CaHPO_4 + H^+ \leftrightarrow Ca^{2+} + H_2PO_4^-$	0.30
$CaHPO_4 \leftrightarrow Ca^{2+} + HPO_4^{2-}$	-6.90
$CaHPO_4 + HCO_3^- + H_2O \leftrightarrow CaCO_3 + HPO_4^{2-} + H^+ + H_2O$	-8.82
Brushite (dicalcium phosphate dihydrate, DCPD)	
$CaHPO_4 \bullet 2H_2O + H^+ \leftrightarrow Ca^{2+} + H_2PO_4^- + 2H_2O$	0.63
$CaHPO_4 \bullet 2H_2O \leftrightarrow Ca^{2+} + HPO_4^{2-} + 2H_2O$	-6.57
$CaHPO_4 \bullet 2H_2O + HCO_3^- \leftrightarrow CaCO_3 + HPO_4^{2-} + H^+ + 2H_2O$	-8.49
Calcium Carbonates	
Calcite: $CaCO_3 + 2H+ \leftrightarrow Ca^{2+} + HCO_3^-$	1.93
Aragonite: $CaCO_3 + 2H+ \leftrightarrow Ca^{2+} + HCO_3^{--}$	2.15
Dolomite: $CaMg(CO_3)_2 + 2H^+ \leftrightarrow Ca^{2+} + Mg^{2+} + 2HCO_3^-$	2.82

Ion activity products (Tables 5-7 thru 5-9) were based on mean chemical concentrations at various times for each of the treatments (Tables 5-1 thru 5-4). Solubility diagrams were developed for selected Ca-P minerals on which empirically derived phosphate (either $H_2PO_4^-$ or HPO_4^{2-} depending on solution pH) activities were plotted (Fig. 5-4). Points lying above a mineral line imply supersaturation with regard to that mineral. The shape and position of the solubility isotherms for each mineral phase depends on reactons involved in a given portion of the diagram. Controlling reactions (Table 5-6) are a function of pH, and $H_2PO_4^-$, HPO_4^{2-}, Ca^{2+}, and HCO_3^- activities. The Ca^{2+} activity was fixed at the mean log Ca^{2+} activity of -3.07 ± 0.02 *M* (± SE). Similarly, log HCO_3^- activity was fixed at -2.72 ± 0.12 *M*. The activity of Ca^{2+} under these conditions is controlled by the activity of HCO_3^- at approximately $pH > 7.3$, the theoretical conditions where $CaCO_3$ formation is predicted to occur (Table 5-5). Reactor solutions were supersaturated with respect to hydroxyapatite (HAP) but undersaturated for any other Ca-P (Table 5-7 thru 5-9 and Fig. 5-4). This suggests that conditions exist such that a Ca-P mineral with stoichiometry similar to that of HAP could theoretically form under these conditions. If HAP exists in the system then it is expected to remain a stable phase. Fig. 5.4 also suggests that formation of Ca-P minerals is limited by the P activity. The molar ratio of Ca to P is higher for HAP than for any of the other Ca-P minerals shown.

The IAPs determined for the carbonates; calcite, aragonite, and dolomite, predict that the bulk water (Day 0), and water incubated under CO_2-enriched air were undersaturated for calcite and aragonite (Table 5-4 thru 5-6). All samples collected under CO_2-free air additions were supersaturated with regard to calcite and aragonite. All solutions were supersaturated with respect to dolomite except for the bulk water (Day 0) and Treatment I day 17.9 (I17.9) and is probably due to limited CO_3^- activity.

Conditions predict that HAP, an HAP-like solid phase, or a $CaCO_3$ mineral should have formed during high pH conditions of Treatments II and III and thus should remove DRP from solution as occurred during the high pH phase of Treatment I. A solid

Table 5-7. Mean (n = 3) log ion activity products (log IAP) calculated for Treatment I water sampled at selected times. Log K^1 = solubility constant when balanced with $H_2PO_4^-$, log K^2 = solubility constant when balanced with HPO_4^{2-}, log K^3 = solubility constant when reaction balanced with HCO_3^-. Conditions where log IAP > log K implies saturation with regard to a mineral phase. Selection of log K is dependent upon solution pH (see Fig. 5-4). Carbonate reactions are balanced with HCO_3^-.

Mineral		Bulk	CO_2-free Air				3% CO_2
		Day 0	Day 6	Day 8	Day 13.5		Day 17.9
	log K^3	log IAP				log K^1	log IAP
HAP	-16.74	-8.95	-13.86	-14.21	-13.13	14.46	15.94
B-TCP	-9.98	-10.02	-12.94	-13.22	-12.61	10.18	6.99
OCP	-17.52	-21.10	-24.96	-23.44	-24.71	11.76	5.04
DCP	-8.82	-11.08	-12.02	-12.23	-12.10	0.60	-1.96
DCPD	-8.49	-11.08	-12.02	-12.23	-12.10	0.63	-1.96
	log K						
Calcite	1.93	1.07	2.94	2.99	2.64		1.15
Aragonite	2.15	1.07	2.94	2.99	2.64		1.15
Dolomite	2.82	2.09	5.98	6.07	5.39		2.19

Table 5-8. Mean (n = 3) log ion activity products (log IAP) calculated for Treatment II water sampled at selected times. Log K^1 = solubility constant when balanced with $H_2PO_4^-$, logK^2 = solubility constant when balanced with HPO_4^{2-}, logK^3 = solubility constant when reaction balanced with HCO_3^-. Conditions where log IAP > log K implies saturation with regard to a mineral phase. Selection of log K is dependent upon solution pH (see Fig. 5-4). Carbonate reactions are balanced with HCO_3^-.

Mineral		3% CO_2					CO_2-free Air
		Day 6	Day 8		Day 13.5		Day 26.5
	log K^1	log IAP		log K^2	log IAP	log K^3	log IAP
HAP	14.46	17.81	16.87	-7.14	-3.85	-16.74	-14.15
B-TCP	10.18	8.10	7.54	-4.22	-6.39	-9.98	-13.33
OCP	11.76	6.5	5.75	-9.84	-15.35	-17.52	-25.84
DCP	0.30	-1.61	-1.79	-6.90	-8.96	-8.82	-12.51
DCPD	0.63	-1.61	-1.79	-6.57	-8.96	-8.49	-12.51
	log K						
Calcite	1.93	1.70	1.56		1.47		3.10
Aragonite	2.15	1.70	1.56		1.47		3.10
Dolomite	2.82	3.33	3.11		2.85		6.12

Table 5-9. Mean (n = 3) log ion activity products (log IAP) calculated for Treatment III water sampled at selected times. Log K^1 = solubility constant when balanced with $H_2PO_4^-$, logK^2 = solubility constant when balanced with HPO_4^{2-}, logK^3 = solubility constant when reaction balanced with HCO_3^-. Conditions where log IAP > log K implies saturation with regard to a mineral phase. Selection of log K is dependent upon solution pH (see Fig. 5-4). Carbonate reactions are balanced with HCO_3^-.

Mineral	Alternating (12h) cycles of CO_2-free Air and 3% CO_2							
		Day 6		Day 8		Day 8.5		Day 26.5
	log K^2	log IAP	log K^3	log IAP	log K^1	log IAP	log K^2	log IAP
HAP	-7.14	-0.40	-16.74	-13.83	14.46	16.62	-7.14	-2.95
B-TCP	-4.22	-4.67	-9.98	-12.78	10.18	7.41	-4.22	-5.87
OCP	-9.84	-13.62	-17.52	-24.50	11.76	5.60	-9.84	-14.67
DCP	-6.90	-8.95	-8.82	-11.72	0.30	-1.81	-6.90	-8.80
DCPD	-6.57	-8.95	-8.49	-11.72	0.63	-1.81	-6.57	-8.80
	log K							
Calcite	1.93	2.67		2.77		1.55		1.53
Aragonite	2.15	2.67		2.77		1.55		1.53
Dolomite	2.82	5.28		5.47		3.04		3.02

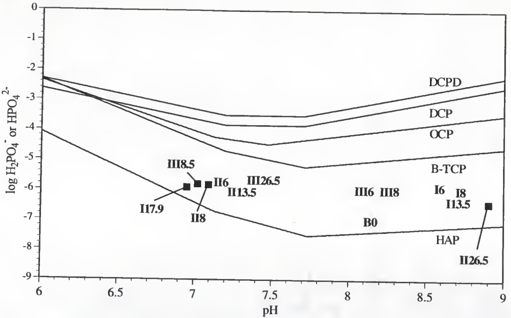

Fig. 5-4. The solubility of selected calcium phosphates and measured solubilities from reaction vessels under varying pH conditions. Calcium activity is fixed at $10^{-3.07}$ M or is fixed by $CaCO_3$ and HCO3- at $10^{-2.72}$ M. Treatment I received CO_2-free air followed by 3% CO_2-containing air. Treatment II received 3% CO_2-containing air followed by CO_2-free air. Treatment III received alternating 3% CO_2 or CO_2-free air on 12 h cycles. Chemical characteristics of water are given in Table 5-1a-d. Calcium phosphates include; $Ca_5(PO_4)_3OH$ (hydroxyapatite, HAP), β-$Ca_3(PO_4)_2$ (β-tricalcium phosphate, B-TCP), $Ca_4H(PO_4)_3 \bullet 2.5\ H_2O$ (octacalcium phosphate, OCP), $CaHPO_4$ (monetite, DCP), $CaHPO_4 \bullet 2\ H_2O$ (brushite, DCPD).

phase was not observed during treatment III and was only found in one vessel of Treatment II which was analyzed to be $CaCO_3$. It took approximately 2.5 days at pH > 8.5 for $CaCO_3$ to precipitate in Treatment I. Although thermodynamically predicted to form, the reaction kinetics of crystal growth inhibited precipitation in Treatment III. The presence of seed crystal increases precipitation reactions. In the field periphyton could increase the rates of precipitation by providing seed crystals. The 12 h pH cycles of Treatment II did not allow enough time for precipitation to occur. If diurnal changes in the field are similar to those of Treatment III then the abiotic complexation of Ca-P from the water column would not provide a stable removal mechanism.

In situ soil porewater chemistry

The chemical composition of the floodwater, BP interstitial water, and soil porewater was modeled, using SOILCHEM, to predict the theoretical stability of Ca-P. Results were similar for both +BP and -BP treatments. In both treatments water was slightly undersaturated for the Ca-P minerals considered (Fig 5-5; Table 5-10).The IAP resulting from the BP and all soil layers were at equilibrium with HAP. This suggests that Ca-P will not form in the water column. The P activity in the water column is just slightly lower than that in the BP and soil. However, the high activity of HCO_3^- (log $HCO_3^- = 10^{-2.4}$ *M*) in the system causes a decrease in Ca activity due to the precipitation of $CaCO_3$. Calcite precipitation begins to affect the Ca-P reactions at pH slightly above 7, but does not depress the ion activity of Ca^{2+} to the extent that the formation of HAP is no longer thermodynamically possible. The soil-BP-water systems of both treatments were supersaturated with respect calcite, aragonite, and dolomite with the exception of the soil below 10 cm depth.which is undersaturated for calcite and aragonite but remains supersaturated for dolomite. Phosphorus bound to $CaCO_3$ as an impurity will be stable in the water, BP, and surface soil.

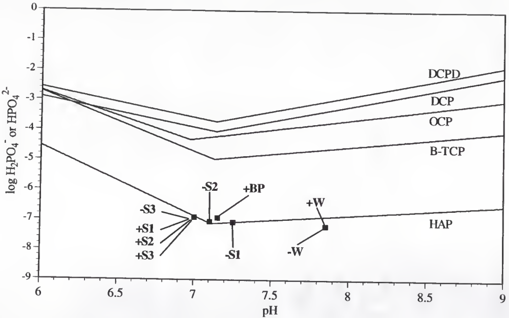

Fig. 5-5. The solubility of selected calcium phosphates and measured solubilities from enclosure porewater equilibrator samples. Enclosures contained intact benthic periphyton (BP) (+) layers, or had BP removed (-). W = water, BP = periphyton interstitial water, S1 - S3 = 0 - 5, 5 - 10, 10 - 15 cm depth, respectively, of soil porewater. Calcium activity was fixed at $10^{-2.8}$ *M* or is fixed by $CaCO_3$ and HCO_3^- at $10^{-2.36}$. Chemical characteristics of the water are given in Table 5-2. Calcium phosphates include; $Ca_5(PO_4)_3OH$ (hydroxyapatite, HAP), β-$Ca_3(PO_4)_2$ (β-tricalcium phosphate, B-TCP), $Ca_4H(PO_4)_3 \bullet 2.5\ H_2O$ (octacalcium phosphate, OCP), $CaHPO_4$ (monetite, DCP), $CaHPO_4 \bullet 2\ H_2O$ (brushite, DCPD).

Table 5-10. Log ion activity products (log IAP) calculated for WCA-2A-217 porewater as collected in porewater equilibrators during September, 1995. log K¹ = equilibrium constant when balanced with $H_2PO_4^-$, log K³ = equilibrium constant when balanced with HCO_3^-. Conditions where log IAP > log K implies saturation with regard to a mineral phase. Selection of log K dependent upon solution pH (see Fig. 5-5). Carbonate reactions are balanced with HCO_3^-.

Treatment/ Mineral		Water	BP		Soil Depth (cm) 0 - 5	5 - 10	10 - 15
	log K³	log IAP		log K¹	log IAP		
+BP							
HAP	-16.74	-16.80	-16.11	14.46	14.61	14.26	14.10
B-TCP	-9.98	-14.60	-13.91	10.18	5.98	5.77	5.68
OCP	-17.52	-27.17	-25.62	11.76	3.34	3.05	2.95
DCP	-8.82	-12.52	-11.71	0.30	-2.64	-2.72	-2.73
DCPD	-8.49	-12.52	-11.71	0.63	-2.64	-2.72	-2.73
	log K						
Calcite	1.93	2.40	2.02		1.99	1.96	1.91
Aragonite	2.15	2.40	2.02		1.99	1.96	1.91
Dolomite	2.82	4.13	3.21		3.17	3.14	3.05

		Water	Soil Depth (cm) 0 - 5	5 - 10	Soil Depth (cm) 10 - 15	
	log K³	log IAP			log K¹	log IAP
-BP						
HAP	-16.74	-17.22	-16.62	-16.66	14.46	14.17
B-TCP	-9.98	-14.92	-14.30	-14.24	10.18	5.70
OCP	-17.52	-27.54	-26.27	-26.07	11.76	2.94
DCP	-8.82	-12.62	-11.97	-11.83	0.30	-2.76
DCPD	-8.49	-12.62	-11.97	-11.83	0.63	-2.76
	log K					
Calcite	1.93	2.51	2.10	1.98		1.90
Aragonite	2.15	2.51	2.10	1.98		1.90
Dolomite	2.82	4.29	3.43	3.20		3.01

X-ray Diffraction of BP and Surficial Soils

Calcite was present in the BP and surficial soils (0 - 2 and 2 - 5 cm depths) analyzed by x-ray diffraction (Fig.5-6). The BP produced the most crystallographic representation of calcite, showing all major peaks. Patterns become less distinct with depth in the soil however, the characteristic calcite peak at 29° 2θ is seen in all samples. A diffraction pattern from an apatite standard was also included showing three characteristic peaks between 30 -35° 2θ. Results from the mineral equilibria modeling predicted stable HAP but this mineral was not seen in the x-ray diffraction patterns. Conversely, predicted calcite was seen in the x-ray patterns.

Conclusions

It was possible to precipitate $CaCO_3$ in Everglades surface water and thus coprecipitate a portion of the DRP. However, this only occurred when the pH was maintained at 8.5 or higher for at least two days. Results of the vessel study suggest that precipitation reactions would not occur under the diurnal variations in field pH. The highest pH attained by alternating gas flow was circa pH = 8.8. Although this is high, it is conceivable that under shallow water conditions , with high photosynthetic activity, the pH could exceed this, especially in close proximity to algal filaments. Increases in pH would facilitate an increasingly rapid precipitation response. Calcareous periphyton calcify to support photosynthesis. This process produces material that can act as seed for crystal growth thus greatly imcreasing precipitation kinetics and the level of supersaturation that must be attained before crystal growth begins. Results of chemical extractions (Chapter 2) and abiotic uptake experiments (Chapters 3 and 4) show that a Ca-P association exists in the field. Thermodynamically, Ca-P of the stoicheometric ratio of HAP would be stable under field conditions.

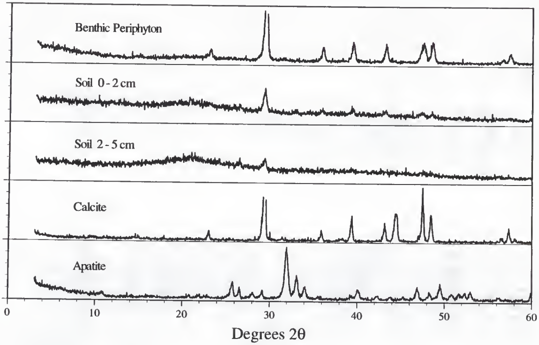

Fig. 5-6. X-ray diffraction patterns for calcite and apatite standards, benthic periphyton, 0 - 2 and 2 - 5 cm layer of peat soil. Characteristic peaks at 29° 2θ were truncated in the calcite standard and benthic periphyton patterns for clarity.

CHAPTER 6
SUMMARY

The periphyton community is an important component of the Everglades ecosystem. Previous research describes the importance of periphyton as a food web base, as the producer of calcitic mud in marl areas or marl layers in peat soils, for being primarily responsible for water column dissolved oxygen production, and as an indicator of environmental nutrient status. Calcareous periphyton is the dominant periphytic type in low nutrient, hardwater areas of the Everglades and is defined by Browder et al. (1994) as having a substantial (> 15%) calcite component and a dominance of the cyanobacterial species *Schizothrix calcicola* (Ag.) Gom. and *Scytonema hofmannii* Ag. Studies have shown increased loading of P to the surface water of the Everglades has caused shifts in the taxonomic composition of the periphyton. Important to the relationship between P and periphyton is the effects calcareous periphyton has on the physicochemical conditions of the water as they influence the P cycle. The periphyton catalyzed precipitation of calcite may influence the bioavailability of P and be responsible for maintaining low ambient water P concentrations in unimpacted Everglades.

This research was conducted to determine important aspects of the Everglades P cycle as influenced by periphytic activity. The objective of this research was to determine the influence of periphyton on P retention in a freshwater wetland. Specific objectives include:

- Determine the quantity and form of P stored in three types of periphyton, water, and soil. It is hypothesized that the quantity of P stored in periphyton depends on periphytic type, and that periphytic $CaCO_3$ production will increase the inorganic P retention capacity of the peat soil.

- Determine the P uptake kinetics of periphyton and partition uptake into biotic and abiotic processes. Phosphorus uptake should be rapid in the P limited interior of WCA-2A. Since periphyton contains both autotrophic and heterotrophic organisms both inorganic and organic P should be utilized. It is also hypothesized that the $CaCO_3$ matrix of the periphyton will adsorb inorganic P thus providing abiotic uptake.
- Determine the influence of benthic periphyton on P flux between soil porewater and the overlying floodwater. It is hypothesized that the activity of benthic periphyton reduces P flux from soil porewater at ambient concentrations. Under enriched conditions, demand for P by benthic periphyton should result in faster uptake as compared to periphyton-less soil. Phosphorus uptake by benthic periphyton, being a function of autotrophic and heterotrophic organisms, should occur under light and dark conditions.
- Determine the physicochemical conditions in the watercolumn which lead to P retention in stabile mineral forms. It is hypothesized that high pH conditions cause precipitation of $CaCO_3$ and coprecipitation of P and that high CO_2 causes dissolution of the solids.
- Assess in situ ion activities and physicochemical conditions to maintain stabile mineral forms.

The field site, designated 217, chosen for sample collection and in situ experiments was in the unimpacted interior of Water Conservation Area 2A. Typical attributes of this site are; low water P concentrations (approx. 10 μg TP L^{-1}), high alkalinity, peat soil, and prolific growths of benthic, epiphytic, and floating forms of calcareous periphyton.

Important aspects of P cycling in periphyton dominated, hardwater areas of the Everglades are depicted in Fig. 6-1. Three main compartments are considered; water, benthic periphyton, and surficial soil. Dissolved reactive P is represented as HPO_4^{2-}

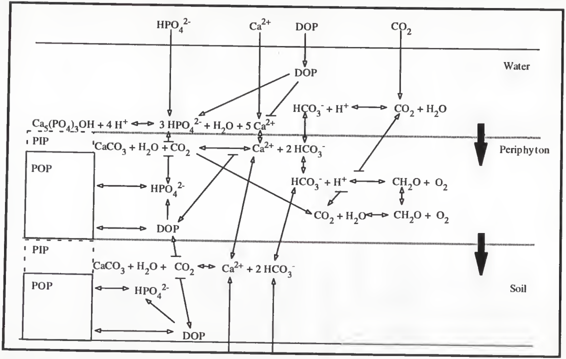

Fig. 6-1. Phosphorus cycle in periphyton dominated, hardwater wetlands.

because this is the dominant form of P over most of the system's pH range (pH = 7 - 9). Inputs come from the atmosphere (HPO_4^{2-} and CO_2), with influent floodwater (HPO_4^{2-}, Ca^{2+}, dissolved organic P, DOP, HCO_3^-, etc.), or from groundwater (Ca^{2+}, HCO_3^-). Soluble ions can diffuse across compartment boundaries in response to concentration gradients.

The periphyton can be separated into three forms based on the position in the water column. Floating periphyton usually exists at or near the floodwater surface and is often associated with the floating macrophyte *Utricularia* spp. Epiphytic periphyton preferentially colonizes the submerged, dead stems of emergent macrophytes, particularly sawgrass (*Cladium jamaicense* Crantz). Benthic periphyton forms a thick (4 - 12 cm) carpet covering the soil surface. The water column position determines the P sources available to a periphyton type. Where floating and epiphytic periphyton derive P nutrition from the water column, benthic periphyton is able to attain P from the floodwater and soil porewater. Benthic periphyton also receives inputs of detrital material (and P contained within) originating as epiphytic periphyton, floating periphyton, and macrophytes falling from the water column. This results in benthic periphyton having twice the total P content of the other two forms.

Periphyton contains both autotrophic and heterotrophic microorganisms and is capable of removing HPO_4^{2-} and dissolved organic P (DOP) from the floodwater (and for benthic periphyton from the porewater) through biological assimilation forming particulate organic P (POP). Uptake at concentrations above ambient (5 μg DRP L^{-1}) typically followed first-order kinetics with uptake rate increasing with initial P concentration. At ambient initial P, there was no net change in solution concentration suggesting that periphyton is in equilibrium with solution P and net community influx equals efflux. Phosphorus uptake generally occurs at a faster rate for epiphytic periphyton when compared to benthic periphyton. Development of Michaelis-Menten parameters showed that epiphytic periphyton had a greater maximum uptake rate (V_{max})

which means it has a greater capacity to remove P from water at elevated P concentrations when compared to benthic periphyton. Benthic periphyton reaches half-maximal uptake rate at a lower solution P concentration (lower K_m) than epiphytic periphyton, meaning benthic periphyton has a higher affinity for P at low concentrations. Dissolved reactive P levels in the interior of WCA-2A are always below K_m and therefore uptake rates are always below V_{max}. Phosphorus uptake rates by periphyton are limited by the available P substrate. Partitoning studies showed that 80 - 95 % of total P uptake was due to biological processes on a short-term basis. As initial P concentration increased the proportion of uptake that was biotic increased.

Autotrophic members of benthic periphyton utilize CO_2 in photosynthesis to produce organic matter. When CO_2 becomes limiting, calcareous cyanobacteria use HCO_3^- to continue photosynthesis, a process that is accompanied by calcification. The $CaCO_3$ produced during calcification forms encrustations on the surface of algal filaments and can account for 20 - 50% of periphyton total dry weight. Inorganic P can become adsorbed to the surface of the $CaCO_3$ crystals and eventually becomes incorporated into the matrix as crystal growth continues. Conditions in the high pH environment that accompanies photosynthetic activity thermodynamically favor the chemical precipitation of mineral forms of Ca-P. The removal of P from the water column by reactions of Ca^{2+} and $CaCO_3$ associated with the periphyton surface is considered abiotic uptake. The P abiotically incorporated in this manner becomes part of the particulate inorganic P (PIP) component of benthic periphyton. Phosphorus uptake by benthic periphyton is therefore a function of both biotic and abiotic processes although biotic uptake dominates on a short-term basis.

Heterotrophic decomposition of periphyton material releases P from particulate organic P forming dissolved organic P which can be directly recycled or can undergo hydrolysis to HPO_4^{2-} which is again incorporated either biotically or abiotically. The hydrolysis of dissolved organic P in solution was shown to be rapid ($>0.7\ \mu M\ \mathrm{min}^{-1}$).

Considering the P limited status of the interior Everglades, the high biological activity in the benthic periphyton layer, and the close proximity of autotrophic and heterotrophic organisms it seems likely that internal recycling of P is intense and little P would be released to the water during periods of normal growth.

Respiration and the aerobic decomposition of organic matter consumes O_2 and generates CO_2. Excess CO_2 is released to the water where it replenishes the HCO_3^- consumed during photosynthesis. The increase in CO_2 due to respiration causes a decrease in pH which affects the stability of $CaCO_3$ or Ca-P minerals. Most (80%) of P coprecipitated with $CaCO_3$ at $pH > 8.5$ returned to solution within 12h at $pH = 7$ and high CO_2.

Heterotrophic organisms consume O_2 causing its depletion in lower BP layers and in the soil. Decomposition occurs anaerobically when O_2 is depleted. Anaerobic decomposition is slow when compared to aerobic decomposition. Under anaerobic conditions organic materials accumulate at the soil surface leading to long-term peat accretion. Respiration in the soil layer is shown consuming oxygen however, a number of other electron acceptors including NO_3^-, Mn^{4+}, Fe^{3+}, and SO_4^{2-} would be utilized in organic matter decomposition under anaerobic conditions.

The benthic periphyton layer is the primary source of organic material, $CaCO_3$, and P accreting on the soil surface as evidenced by the similar total P concentrations of these two layers. The rate of peat accretion in benthic periphyton dominated areas is very slow (0.7 mm yr^{-1}). Browder et al. (1994) described these systems as having relatively high gross primary productivity but that most of the production is used by the photosynthesizing organisms and decomposers. This results in very low system net primary production which is consistent with the low accretion rates. In terms of P accretion around 25 mg total P m^{-2} yr^{-1} is being added to the soil. Approximately 50% of the added P is in resistant organic forms, the P fraction that comprises the majority of P storage in the peat. The proportion of inorganic P is higher in the surface soil and decreases with depth. Much of this P is associated with $CaCO_3$ which showed a similar trend.

Although hydroxyapatite is a thermodynamically predicted stable mineral P form in benthic periphyton and soil it is likely that abiotically incorporated P is held as an impurity in $CaCO_3$. Mineral forms of Ca-P have not been observed in BP or the surface 0-5 cm of peat. However, mineral calcite was identified in these layers.

Organic matter in the surface soil is subjected to microbial decomposition which causes the release of dissolved organic P with subsequent mineralization to HPO_4^{2-}. Phosphorus is recycled in this layer in ways similar to the benthic periphyton layer. If excess HPO_4^{2-} is released by soil decomposition processes it will diffuse into and be utilized by the overlying benthic periphyton layer.

Decomposition in the benthic periphyton and soil is reduced under anaerobic conditions however, shallow interior areas of the Everglades often dry completely, usually on a seasonal basis. During these periods, the organic matter in benthic periphyton and surface soils is rapidly oxidized. The P released is incorporated into the soil microbes (POP) or becomes part of the soil particulate inorganic P. Phosphorus abiotically bound and $CaCO_3$ in the benthic periphyton layer are added to the soil surface. The $CaCO_3$ accumulates in the soil increasing the soil's P retention capacity. Soil without a functioning benthic periphyton layer can initially remove floodwater P at rates equal to or faster than the benthic layer. Soil microbes and adsorption to mineral surfaces are responsible for this uptake. The soil capacity for uptake is limited and continued loading quickly saturates the surface layers of soil and its ability to maintain low P water concentrations. Soils were shown to have diminished P removal capacity after receiving 40 mg DRP m^{-2} whereas intact benthic periphyton layers received twice this load and exhibited no reduction in uptake capacity.

This research has shown that P cycling in unimpacted periphyton dominated Everglades is largely controlled by biological uptake on the short-term and that calcification leads to a long-term incorporation of Ca-P in surficial soils. Increased calcification would encourage P movement into this mineral storage. Calcification is promoted when HCO_3^- is used for photosynthesis because CO_2 is limited. Favorable conditions would include shallow water depths, providing a limited CO_2 source, and a thin and highly autotrophic BP layer.

Consequences of the loss of a functioning periphyton system would result in a reduced capacity to remove water column P additions via biotic uptake, and a diminished capacity for soil retention of P, especially for the long-term. More importantly, the system would loose its buffering capacity to maintain low water column P concentrations. These conditions would result in elevated surface water P concentrations which cause alterations in community structure.

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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